ATTACHMENTS

Statistics suggest BSE now 'Europe-wide'

Paris. Europe's veterinary surgeons have urged the European Union (EU) to introduce and enforce measures to ensure that bovine spongiform encephalopathy (BSE) cannot enter the human food chain. Their call comes as new analyses suggest that the incidence of BSE in continental Europe is much higher than officially reported.

EU member states in continental Europe have declared a total of around 50 cases of BSE (see table, right). But some researchers estimate that these countries ought to have declared more than 2,000 cases, given that they imported large quantities of breeding cattle and potentially contaminated meat and bone meal from the United Kingdom (see *Nature* **381**, 544; 1996).

One explanation for this discrepancy, say observers, is that farmers may have been discouraged from reporting cases because many countries slaughter herds where BSE has occurred without adequately compensating farmers.

These estimates of the number of BSE cases in continental Europe are nonetheless an order of magnitude lower than in the United Kingdom, where around 160,000 cases have been reported. It follows that any impact on human health would be correspondingly lower, even if it was established that the agent that causes BSE can pass to humans and cause Creutzfeldt-Jakob disease (CJD). Experts also point out that most of the BSE cases in continental Europe will already have entered the food chain.

Nevertheless, at a meeting in Paris last month, the Federation of Veterinarians of Europe (FEV) acknowledged the BSE crisis is now a "European problem" — and not just a British one — according to one official present. Tougher European measures to protect public health are needed, he argues.

He points out that, until last week, when France banned the human consumption of specified bovine offals (SBO) — the most infective parts, such as brain and spinal cord — (see page 5), the United Kingdom had been the only EU country to have done so. (Switzerland, which is not a member of the EU, had introduced a similar ban.)

To reassure consumers, "it must be demonstrated beyond any doubt that BSE cannot enter the food chain", says Francis Antony, president of FEV and the British Veterinary Association's spokesman on BSE. In a statement last week, FEV called on the EU to "design, apply and enforce" a comprehensive food hygiene programme "from the stable to the table".

"Consumer perception and the scientific reality of food safety are too far apart," says one FEV official. "The key issue is about keeping brain and spinal cord out of the food chain." Indeed, the FEV statement seems a thinly veiled attack on what one official says is the EU's excessive focus on the culling of UK cattle.

Although culling will precipitate the decline of the epidemic in cattle, it will have little direct impact on human health, argues one FEV official. Eliminating risk to public health requires a ban on the human consumption of SBOs and strict enforcement of deboning and denerving procedures in abattoirs to eliminate infective tissues.

The EU also needs to ensure that adequate resources are made available to enforce any new regulations, says one FEV official. The failure of the United Kingdom to police its early feed and SBO bans has shown that "you can ban things until you are blue in the face", but without proper enforcement this has little impact.

The revised estimates of cases of BSE in mainland EU states come from analyses of UK exports of animal feed and live cattle. Experts predict that imported feed may have resulted in several hundred cases of BSE. But the total is difficult to estimate precisely because the final destination and use of feed are often unknown, while the relationship between tonnage and infectivity is difficult to establish, according to John Wilesmith, of the UK Ministry of Agriculture's Central Veterinary Laboratory in Weybridge.

Better estimates may come from analysis of UK exports of breeding cattle. One group of researchers has estimated, for example, that the United Kingdom exported 57,900 pure-bred breeding bovines between 1985 and 1990, the latter being the year after the EU banned imports of breeding animals older than six months.

One of the scientists, B. E. C. Schreuder from the DLO-Institute for Animal Science and Health in Lelystad, the Netherlands, says that, statistically, around 1,700 of these animals would be expected to have developed BSE.

Such estimates must be taken with caution because of the assumptions they involve, says Schreuder. His estimates of total exports are somewhat higher than records of exports for "pure-bred breeding bovines" (see figure, below left) provided by Eurostat — the body that supplies the European Commission with statistics — as he has also included other export categories such as "bulls not intended for slaughter".

Number of declared cases of BSE worldwide (May 1996)

UK	158,866
Switzerland	211
Eire	125
France	18
Portugal	30*

*excluding 6 cases in imported animals

World Animal Health Organization

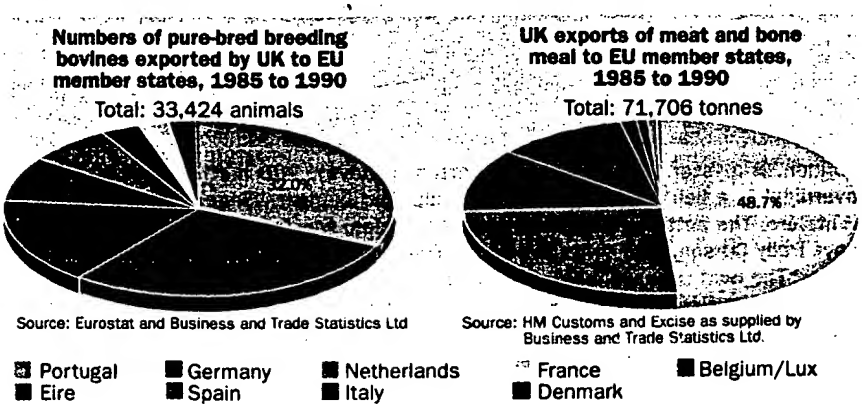
Nonetheless, Schreuder is confident that their estimate of BSE cases in continental Europe attributable to cattle exports is of the right order of magnitude. He says it is possible to identify the individual member states most at risk — Portugal, which has declared just 30 cases of BSE, is expected to top this list.

Schreuder says that his warning of underreporting is mainly aimed at countries where lack of regulation might yet allow recycling of BSE and new outbreaks. Wilesmith adds that better data on true BSE levels in continental Europe will be essential to studying the epidemiology of any cases of the new variant of CJD that arise there. The evidence for higher levels of BSE on the European mainland also underlines the need for an effective European CJD surveillance network, he says (see *Nature* **381**, 453; 1996).

Wilesmith asserts that "we were lucky there were not BSE epidemics elsewhere". The factors that led the United Kingdom to be worst affected, he says, probably included the world's highest ratio of sheep to cattle (45 million to 12 million), a high prevalence of scrapie, and the use of high proportions of meat and bone meal in cattle feed.

Other countries may have escaped relatively unscathed only because they had more cattle than sheep, low levels of scrapie, or used less meat and bone meal in cattle feed. Indeed, a new report from the UK Institute of Animal Health suggests that the procedures used in many rendering plants throughout Europe would have been insufficient to inactivate the agent that causes BSE.

Declan Butler



Supercomputer 'dumping' dispute heats up

Washington. A vote last week in the US House of Representatives has raised the political stakes of a pending decision by the National Science Foundation (NSF) whether to allow the leasing of Japanese-made supercomputers to the National Center for Atmospheric Research (NCAR).

The University Corporation for Atmospheric Research (UCAR), which runs NCAR for the NSF, expects to conclude negotiations "in the near future" to lease four supercomputers built by Japan's NEC Corporation. The supercomputers would be used to run complex climate models. NSF would have to approve any leasing contract.

But the NSF appropriations bill for 1997 passed in the House last week includes a measure sponsored by two House Democrats — David Obey (Wisconsin) and Martin Sabo (Minnesota) — denying salaries to any NSF official who approves the transaction, if the US Commerce Department ultimately finds that NEC is guilty of price "dumping" (see *Nature* **381**, 723; 1996).

That verdict will be slow in coming. Last week, Cray Research, which lost the leasing bid to NEC, had not yet filed a formal complaint of dumping to the Commerce Department. But the company does intend to file a complaint, according to William Bartolone, vice-president for government operations, after which the department would have 20 days to decide whether to launch an investigation. If it does, even a preliminary verdict could take four months.

The department could decide to investigate the matter on its own, although that rarely happens. In a letter last week, Neal Lane, the NSF director, asked Mickey Kantor, the Commerce Secretary, to notify him if the department plans such a "self-initiated" investigation or if it finds, after reviewing data recently supplied by the NSF, "that its concerns are allayed so that such an investigation is not warranted".

The NSF has twice asked UCAR to look closely at the NEC bid and to confirm that it does not include noncompetitive practices. In a statement last week, UCAR said that, after hiring outside consultants to review the bid, it has concluded that "the best and final offer of [NEC] is fair".

The NSF clearly believes, too, that right is on its side. Lined up against it, however, are protectionist forces in Congress. The American Federation of Labor and the presidential gadfly Ross Perot were only two of those who weighed in last week to encourage the House to squash the NEC supercomputer deal.

A recent congressional vote on a Japanese shipbuilding contract, which went in favour of protectionism, was said to be a factor in a decision by Jim Kolbe (Republican, Arizona) to withdraw his amendment deleting the Obey–Sabo clause.

Kolbe did, however, secure a promise that the matter will be debated again before the House and Senate finalize the NSF appropriations bill this autumn.

That gives the NSF at least several weeks to decide whether to approve the NCAR deal, and to ponder the wisdom of bucking what has been an unwritten rule among large federal laboratories — that, when it comes to supercomputers, the only way to buy is American. **Tony Reichhardt**

Former head of cancer charity arrested

Paris. **Jacques Crozemarie, who was ousted as chairman of France's biggest medical charity, L'Association pour le recherche contre le cancer (ARC), after a financial scandal earlier this year, was last week arrested and indicted on fraud charges.**

Denis Baumont and Michel Simon, the heads of two companies that are alleged to have enjoyed privileged contracts from ARC were also arrested in police swoops and remanded in custody; the two companies are alleged to have overcharged the charity by substantial amounts and taken excessive commissions (see *Nature* 379, 103; 1996). □

French action aims to quell BSE fears

Paris. France moved swiftly last week to respond to the advice of its scientific advisory committee on bovine spongiform encephalopathy (BSE), spurred by criticism of its failure in early May to respond to the committee's suggestion to act as if BSE can be passed to humans.

Alain Juppé, the prime minister, announced that the government would implement to the letter a series of recommendations submitted by the committee just a few hours earlier. These include the following.

- Meat and bone meal should be manufactured only from abattoir wastes considered fit for human consumption, and the practice of processing carcasses of farm and domestic animals should be discontinued.
- There should be a ban on the use of all central nervous system tissues of ruminants both in meat and bone meal and for human consumption. (In April the government banned human consumption of such tissues from animals born before August 1991.)
- France should ask for its ban on the import of meat and bone meal from the United Kingdom to be extended to all members of the European Union (EU).
- There should be strict separation of feed used for ruminants and that used for other animals, enforced by new tracking systems and better controls.

The high political profile given to the announcement appears to have been part of an attempt by the government to restore public faith in its commitment to openness about BSE. This was damaged last month when the newspaper *Le Monde* revealed that the BSE advisory committee had advised the government on 9 May to act as if BSE could be transmitted to humans but that the government had decided to keep the report secret until 7 June.

Alain Juppé (right) announces the French measures on BSE, with agriculture minister Philippe Vasseur.

The revelations were particularly embarrassing in that they established that Jacques Chirac, the French president, was aware of this recommendation when, in mid-May he promised his support to John Major, the British prime minister, for a partial lifting of the EU ban on UK beef products.

The French government's concern to avoid charges of withholding information from the public is also said to have led it to require a group of researchers to hold a press conference last month about a paper, unpublished at the time (and now published in *Nature* **381**, 743–744; 1996) reporting that macaques inoculated with infected cattle brain developed similar pathological symptoms to humans with the new variant of Creutzfeldt-Jakob disease.

- The French consumer association UFC-Que Choisir announced last week that it will bring legal proceedings against 'X' for the marketing of potentially contaminated meat and bone meal imported from the United Kingdom (see *Nature* **381**, 544; 1996). Jacques Toubon, the justice minister, said that he was considering taking similar action. **Declan Butler**

Molecular Biology of Prion Diseases

STANLEY B. PRUSINER

Prions cause transmissible and genetic neurodegenerative diseases, including scrapie and bovine spongiform encephalopathy of animals and Creutzfeldt-Jakob and Gerstmann-Sträussler-Scheinker diseases of humans. Infectious prion particles are composed largely, if not entirely, of an abnormal isoform of the prion protein, which is encoded by a chromosomal gene. A posttranslational process, as yet unidentified, converts the cellular prion protein into an abnormal isoform. Scrapie incubation times, neuropathology, and prion synthesis in transgenic mice are controlled by the prion protein gene. Point mutations in the prion protein genes of animals and humans are genetically linked to development of neurodegeneration. Transgenic mice expressing mutant prion proteins spontaneously develop neurologic dysfunction and spongiform neuropathology. Understanding prion diseases may advance investigations of other neurodegenerative disorders and of the processes by which neurons differentiate, function for decades, and then grow senescent.

PRIONS ARE INFECTIOUS PATHOGENS THAT DIFFER FROM bacteria, fungi, parasites, viroids, and viruses, both with respect to their structure and with respect to the diseases that they cause (*1*). Molecular biological and structural studies of prions promise to open new vistas into fundamental mechanisms of cellular regulation and homeostasis not previously appreciated. Kuru, Creutzfeldt-Jakob disease (CJD), and Gerstmann-Sträussler-Scheinker syndrome (GSS) are all human neurodegenerative diseases that are caused by prions and are frequently transmissible to laboratory animals (*2*). Familial CJD and GSS are also genetic disorders. Individuals at risk can often be identified decades in advance of central nervous system (CNS) dysfunction (*3, 4*), yet no effective therapy exists to prevent these lethal disorders.

In addition to the three prion diseases of humans, four disorders of animals are included in the ensemble of prion diseases. Scrapie of sheep and goats is the most studied of the prion diseases. Bovine spongiform encephalopathy (BSE), transmissible mink encephalopathy, and chronic wasting disease of captive mule deer and elk are all thought to result from the ingestion of scrapie-infected animal products. BSE threatens the beef industry of Great Britain (*5*) and possibly other countries; the production of pharmaceuticals (*6*) involving cattle is also of concern. Control of sheep scrapie in many countries is a persistent and vexing problem (*7*).

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Since 1986, more than 28,500 cattle have died of BSE in Great Britain (*5*). Many investigators contend that BSE, often referred to as "mad cow disease," resulted from the feeding of dietary protein supplements derived from rendered sheep offal infected with scrapie to cattle, a practice banned since 1988 (*5*). It is thought that BSE will disappear with the cessation of feeding rendered meat and bone meal, as has been the case in kuru of humans, confined to the Fore region of New Guinea and once the most common cause of death among women and children. Kuru has almost disappeared with the cessation of ritualistic cannibalism, suggesting that kuru was transmitted orally, as proposed for BSE.

The Prion Hypothesis

The unusual biological properties of the scrapie agent were first recognized in studies with sheep (*8*). The experimental transmission of scrapie to mice (*9*) gave investigators a convenient laboratory model that provided information on the nature of the unusual infectious pathogen that causes scrapie (*10, 11*). Yet progress was slow because quantitation of infectivity in a single sample required housing 60 mice for 1 year before accurate scoring could be accomplished (*9*).

The development of a more rapid and economical bioassay for the scrapie agent in Syrian golden hamsters accelerated purification of the infectious particles (*12, 13*). Partial purification led to the discovery that a protein is required for infectivity (*14*), in agreement with earlier studies that raised the possibility that protein might be necessary (*15*). Procedures that modify nucleic acids did not alter scrapie infectivity (*1*). Other investigators found that scrapie infectivity resisted inactivation by both ultraviolet and ionizing radiation (*10*); these results prompted speculation that the scrapie pathogen might be devoid of nucleic acid—a postulate dismissed by most scientists. In addition to ultraviolet irradiation, reagents specifically modifying or damaging nucleic acids, such as nucleases, psoralens, hydroxylamine, and Zn^{2+} ions, do not alter scrapie infectivity in homogenates (*1*), microsomal fractions (*1*), purified prion rod preparations, or detergent-lipid-protein complexes (*16, 17*).

On the basis of these findings, I introduced the term "prion" to distinguish the proteinaceous infectious particles that cause scrapie, CJD, GSS, and kuru from both viroids and viruses (*1*). Hypotheses for the structure of the infectious prion particle included the following: (i) proteins surrounding a nucleic acid that encodes the proteins (a virus), (ii) proteins associated with a small polynucleotide, and (iii) proteins devoid of nucleic acid. Mechanisms postulated for the replication of infectious prion particles included those used by viruses, the synthesis of polypeptides in the absence of nucleic acid template, and posttranslational modifications of cellular proteins. Subsequent discoveries have narrowed hypotheses for both prion structure and the mechanism of replication.

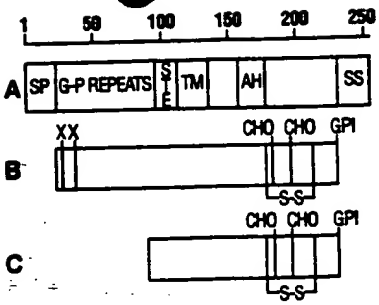

Fig. 1. Structural features of the Syrian golden Ha prion protein. Codon numbers are indicated at the top of the figure. (**A**) NH_2-terminal SP of 22 amino acids is removed during biosynthesis (*20, 23*). The NH_2-terminal region contains five Gly-Pro-rich (G-P) octarepeats and two hexarepeats; between codons 96 and 112 a domain controlling PrP topology is designated as the stop-transfer effector (STE) (*52*); codons 113 to 135 encode a transmembrane (TM) α-helix; codons 157 to 177 encode an amphipathic helix (AH) (*52*); and codons 232 to 254 encode a hydrophobic signal sequence (SS) that is removed when a GPI anchor is added (*24*). (**B**) Unknown modifications (X) of the arginine residues at codons 25 and 37 in PrP^{Sc} and at least codon 25 in PrP^{C} result in a loss of the arginine signal in the Edman degradation, but these modifications are inconsistently reported (*23*). Both PrP isoforms contain a disulfide (S–S) bond between Cys^{179} and Cys^{214} (*23*); asparagine-linked glycosylation (CHO) occurs at residues 181 and 197 (*25*), and a GPI anchor is attached to Ser^{231} (*24*). (**C**) PrP 27-30. This molecule is derived from PrP^{Sc} by limited proteolysis that removes the NH_2-terminal 67 amino acids and leaves a protease-resistant core of 141 amino acids (*20, 21*).

Discovery of the Prion Protein

Progress in the study of prions and the degenerative diseases of the CNS that they cause was accelerated by the discovery of a protein designated prion protein (PrP) (*18*). In subcellular fractions from hamster (Ha) brain enriched for scrapie infectivity, a protease-resistant protein of 27 to 30 kD, designated PrP 27-30, was identified; it was absent from controls. Purification of PrP 27-30 to homogeneity allowed determination of its NH_2-terminal amino acid sequence (*19*), which in turn permitted the synthesis of isocoding mixtures of oligonucleotides that researchers used to identify PrP complementary DNA (cDNA) clones (*20, 21*). PrP is encoded by a chromosomal gene and not by a nucleic acid in the infectious scrapie prion particle (*20*). Levels of PrP messenger RNA (mRNA) remain unchanged throughout the course of scrapie infection—an observation that led to the identification of the normal PrP gene product, a protein of 33 to 35 kD, designated PrP^{C} (*20*). PrP^{C} is protease-sensitive, whereas PrP 27-30 is the protease-resistant core of a 33- to 35-kD disease-specific protein, designated PrP^{Sc}.

Sequencing of molecular clones recovered from cDNA libraries that had been constructed from mRNA isolated from scrapie-infected Syrian Ha and mouse (Mo) brains showed that the Ha and MoPrP cDNAs encode proteins of 254 amino acids (Fig. 1) (*20, 21*). Identical sequences were deduced from genomic clones derived from DNA of uninfected, control animals (*20*). Human PrP consists of 253 amino acids (*22*). Signal peptides (SPs) of 22 amino acids at the NH_2-terminus are cleaved during the biosynthesis of Ha and MoPrPs in the rough endoplasmic reticulum (*23*). Twenty-three amino acids are removed from COOH-terminus of HaPrP on addition of a glycoinositol phospholipid (GPI) anchor (*24*). Two asparagine-linked oligosaccharides are attached to sites in a loop formed by a disulfide bond (*23, 25*). Limited proteolysis of PrP^{Sc} removes ~67 amino acids from its NH_2-terminus to produce PrP 27-30 (*19, 20*). Neither gas-phase sequencing nor mass spectrometric analysis of PrP 27-30 have revealed any amino acid differences between the sequences thus determined and that deduced from the translated sequence of molecular clones (*26*). The covalent structure of PrP^{Sc} remains uncertain because purified fractions contain ~10^5 PrP 27-30 molecules per ID_{50} unit (*18*). (One ID_{50} unit is the infectious dose at which 50% of the animals develop scrapie.) If <1% of the PrP^{Sc} molecules contained an amino acid substitution or posttranslational modification that conferred scrapie infectivity, our methods would not detect such a change (*27*).

Infectious Prion Particles

Information on PrP^{Sc} in prion diseases indicates that prions are composed of PrP^{Sc} molecules (Table 1). Although some investigators contend that PrP^{Sc} is merely a pathologic product of scrapie infection and that PrP^{Sc} coincidentally purifies with the "scrapie virus" (*28*), there are few data to support this view. No infective fractions containing <1 PrP^{Sc} molecule per ID_{50} unit have been found; such a result would indicate that PrP^{Sc} is not required for infectivity. Some investigators report that PrP^{Sc} accumulation in hamsters occurs after the synthesis of many infective units (*29*), but these results have been refuted (*30*). The discrepancy appears to be due to comparisons of infectivity in crude homogenates with PrP^{Sc} concentrations measured in purified fractions.

The search for a component in the prion particle other than PrP has focused on a nucleic acid because the existence of such a component would readily explain different isolates or strains of infectivity (*31*). Specific scrapie isolates characterized by distinct incubation times retain this property when repeatedly passaged in mice or hamsters (*31*). Other factors modulating scrapie incubation times include PrP gene expression, murine genes linked to PrP (*Prn-i* and *Sinc*), dose of inoculum, route of inoculation, and the genetic origin of the prion inoculum. A scrapie-specific nucleic acid has not been found with reagents that modify or hydrolyze polynucleotides, with molecular cloning procedures, or with physicochemical techniques (*16, 17, 32*). Although available data do not permit exclusion of a scrapie-specific polynucleotide (*27*), its existence seems unlikely. That prions might contain noncovalently bound cofactors, such as peptides, oligosaccharides, fatty acids, sterols, or inorganic compounds, deserves consideration.

Table 1. Evidence that PrP^{Sc} is a major and necessary component of the infectious prion.

1) Copurification of PrP 27-30 and scrapie infectivity by biochemical methods. Concentration of PrP 27-30 is proportional to prion titer (*18, 23*).
2) Kinetics of proteolytic digestion of PrP 27-30 and infectivity are similar (*18*).
3) Copurification of PrP^{Sc} and infectivity by immunoaffinity chromatography. α-PrP antisera neutralization of infectivity (*38*).
4) PrP^{Sc} detected only in clones of cultured cells producing infectivity (*50a*).
5) PrP amyloid plaques are specific for prion diseases of animals and humans (*34*). Deposition of PrP amyloid is controlled, at least in part, by the PrP sequence (*71*).
6) Correlation between PrP^{Sc} (or PrP^{CJD}) in brain tissue and prion diseases in animals and humans (*82*).
7) Genetic linkage between MoPrP gene and scrapie incubation times (*55, 56*). PrP gene of mice with long incubation times encodes amino acid substitutions at codons 108 and 189, as compared to mice with short or intermediate incubation times (*41*).
8) Syrian HaPrP transgene and scrapie PrP^{Sc} in the inoculum govern the "species barrier," scrapie incubation times, neuropathology, and prion synthesis in mice (*71, 72*).
9) Genetic linkage between human PrP gene mutation at codon 102 and development of GSS (*3*). Association between codon 200 point mutation or codon 53 insertion of six additional octarepeats and familial CJD (*4, 62*).
10) Mice expressing MoPrP transgenes with the point mutation of GSS spontaneously develop neurologic dysfunction, spongiform brain degeneration, and astrocytic gliosis (*61*).

PrP Polymers and Amyloid

The discovery of PrP 27-30 in fractions enriched for scrapie infectivity was accompanied by the identification of rod-shaped particles (*18, 33*). The rods are ultrastructurally indistinguishable from many purified amyloids and display the tinctorial properties of amyloids (*33*). These findings were followed by the demonstration that amyloid plaques in prion diseases contain PrP, as determined by immunoreactivity and amino acid sequencing (*34*). Some investigators believe that scrapie-associated fibrils are synonymous with the prion rods and are composed of PrP, even though these fibrils can be distinguished ultrastructurally and tinctorially from amyloid polymers (*35, 36*).

The formation of prion rods requires limited proteolysis in the presence of detergent (*37*). Thus, the prion rods in fractions enriched for scrapie infectivity are largely, if not entirely, artifacts of the purification protocol. Solubilization of PrP 27-30 into liposomes with a retention of infectivity (*17*) demonstrated that large PrP polymers are not required for infectivity and permitted the copurification of PrP^{Sc} and infectivity by immunoaffinity chromatography (*38*).

PrP Gene Structure and Expression

Localization of PrP genes to the short arm of human chromosome 20 and the homologous region of Mo chromosome 2 suggests that PrP genes existed before the speciation of mammals (*39*). Hybridization studies demonstrated <0.004 PrP gene sequences per ID_{50} unit in purified prion fractions, indicating that the gene encoding PrP^{Sc} is not a component of the infectious prion particle (*20*). This feature distinguishes prions from viruses, including those retroviruses that carry cellular oncogenes, and from satellite viruses that derive their coat proteins from other viruses that had previously infected plant cells.

The entire open reading frame of PrP genes is contained in a single exon, eliminating the possibility that variant forms of PrP arise from alternative RNA splicing (*20, 40, 41*), but not excluding such mechanisms as RNA editing or protein splicing (*42*). The two exons of the HaPrP gene are separated by a 10-kb intron: exon 1 encodes a portion of the 5′ untranslated leader sequence, whereas exon 2 encodes PrP and the 3′ untranslated region (*20*). The MoPrP gene is composed of three exons, with exon 3 analogous to exon 2 of the Ha gene (*40*). The promoters of both the Ha and MoPrP genes contain copies of G-C–rich nonamers that may function as a canonical binding site for the transcription factor Sp1 (*43*).

Although PrP mRNA is constitutively expressed in the brains of adult animals (*20*), it is regulated during development. In the septum, PrP mRNA and choline acetyl transferase were found to increase in parallel during development (*44*). In other brain regions, PrP gene expression occurred at an earlier age. The highest concentrations of PrP mRNA are found in neurons (*45*).

Four regions of the open reading frame of the mammalian PrP gene are conserved when the translated amino acid sequences are compared (Fig. 2) (*20–22, 46, 47*). Although the function of PrP^{C} is unknown, the MoPrP sequence is ~30% identical with a molecule found in fractions enriched for the acetylcholine receptor–inducing activity in chickens (*48*).

Synthesis of PrP Isoforms

Pulse-chase experiments with scrapie-infected cultured cells indicate that conversion of PrP^{C} is a posttranslational event (*49*). Although the synthesis and degradation of PrP^{C} are rapid (*49, 50*),

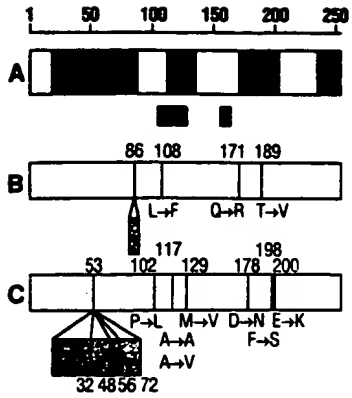

Fig. 2. Genetic map of prion protein open reading frames. Codon numbers are indicated at the top of the figure. (**A**) Four regions conserved among mammalian PrP molecules (hatched) (*20–22, 46, 47*); regions of MoPrP homologous to a molecule found in fractions containing acetylcholine receptor–inducing activity in chickens (black) (*48*). (**B**) Animal mutations and polymorphisms. Two alleles of bovine PrP identified, with one containing an additional octarepeat (stippled) at codon 86; a polymorphism at codon 171 in sheep PrP resulting in the substitution of arginine for glutamine (*46*). Mice with *Prn-p*^{b} genes have long scrapie incubation times and amino acid substitutions at codons 108 (Leu → Phe) and 189 (Thr → Val) (*41*). (**C**) Human PrP mutations and polymorphisms. Octarepeat inserts of 32, 48, 56, and 72 amino acids have been found (*60, 62*). Inserts of 48, 56, and 72 amino acids are associated with familial CJD. Point mutations at codons 102 (Pro → Leu), 117 (Ala → Val), and 198 (Phe → Ser) are found in patients with GSS (*3, 60, 66*). There are common polymorphisms at codons 117 (Ala → Ala) and 129 (Met → Val) (*66, 83*). Point mutation at codons 178 (Asp → Asn) and 200 (Glu → Lys) are found in patients with familial CJD (*4, 64, 66*). Single letter code for amino acids is as follows: A, Ala; D, Asp; E, Glu; F, Phe; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; and V, Val.

the accumulation of PrP^{Sc} is slow and confined to the chase period (Table 2) (*49*). These observations are in accord with studies that show that PrP^{Sc} accumulates in the brains of scrapie-infected animals, yet PrP mRNA concentrations remain unchanged (*20*).

Both PrP isoforms transit through the Golgi apparatus, where their asparagine-linked oligosaccharides are modified and sialic acid is added (*25*). PrP^{C} is presumably transported in secretory vesicles to the external cell surface, where it is anchored by a GPI moiety (*24*). In contrast, PrP^{Sc} accumulates in cells, where it is deposited in cytoplasmic vesicles, many of which appear to be secondary lysosomes (*50a*). Much of the mass added to PrP^{Sc} during posttranslational modification is due to asparagine-linked oligosaccharides, but they are not required for the synthesis of

Table 2. Properties of cellular and scrapie PrP isoforms. Numbers in parentheses after the properties indicate the reference source. PIPLC, phosphatidylinositol-specific phospholipase C. Half-time in hours, $t_{1/2}$.

Property	PrP^{C}	PrP^{Sc}
Concentration in normal Syrian Ha brain (*71*)	~1 to 5 μg/g	
Concentration in scrapie-infected Syrian Ha brain (*71*)	~1 to 5 μg/g	~5 to 10 μg/g
Presence in purified prions (*18, 19, 33*)	–	+*
Protease resistance (*18–20, 33*)	–	+†
Presence in amyloid rods (*33, 34, 37*)	–	+‡
Subcellular localization in cultured cells (*24, 50a*)	Cell surface	Cyto§ vesicles
PIPLC release from membranes (*24*)	+	–
Synthesis ($t_{1/2}$) (*49, 50*)	<0.1	~1 to 3‖
Degradation ($t_{1/2}$) (*49, 50*)	~5	>>24

*Copurification of PrP^{Sc} and prion infectivity demonstrated by two protocols: (i) detergent extraction followed by sedimentation and protease digestion, and (ii) PrP 27-30 monoclonal antibody affinity chromatography. †Limited proteinase K digestion of $HaPrP^{Sc}$ produces PrP 27-30. ‡After limited proteolysis of PrP^{Sc} (PrP 27-30 is produced) and detergent extraction, amyloid rods form; except for length, the rods are indistinguishable from amyloid filaments forming plaques. §PrP^{Sc} is localized primarily in cytoplasmic vesicles. ‖PrP^{Sc} de novo synthesis is a posttranslational process.

protease-resistant PrP in scrapie-infected cultured cells (*51*). This conclusion is based on results with the glycosylation inhibitor tunicamycin and with the expression of recombinant PrP with mutated asparagine-linked glycosylation sites. Experiments with transgenic mice may resolve whether unglycosylated PrP^{Sc} is associated with scrapie infectivity.

Two forms of PrP are found in cell-free translation studies: a transmembrane form that spans the bilayer twice (at the transmembrane and amphipathic helix domains) and a secretory form (Fig. 1) (*52*). The stop-transfer effector domain controls the topogenesis of PrP. That PrP contains a transmembrane domain as well as a GPI anchor poses a topologic conundrum. It seems likely that membrane-dependent events feature in the synthesis of PrP^{Sc}, especially because brefeldin A, which selectively destroys the Golgi stacks, prevents PrP^{Sc} synthesis in scrapie-infected cultured cells (*53*). The association of scrapie infectivity with membrane fractions has been appreciated for many years (*11*); hydrophobic interactions are thought to be responsible for the insolubility of infectious prion particles and for many of the difficulties encountered during attempts to characterize the particles (*13, 17, 54*).

Genetic Linkage of PrP with Scrapie Incubation Times

Studies of PrP genes (*Prn-p*) in mice with short and long scrapie incubation times demonstrated genetic linkage between a *Prn-p* restriction fragment length polymorphism (RFLP) and genes (*Prn-i* and *Sinc*) that modulate the incubation times of the disease (*55–57*). It remains to be established whether *Prn-p*, *Prn-i*, and *Sinc* are all allelic. The PrP sequences of NZW ($Prn\text{-}p^a$) and I/Ln ($Prn\text{-}p^b$) mice with short and long scrapie incubation times, respectively, differ at codons 108 and 189 (Fig. 2) (*41*). Although these amino acid substitutions suggest a congruency of *Prn-p* and *Prn-i*, experiments with $Prn\text{-}p^a$ mice expressing $Prn\text{-}p^b$ transgenes demonstrated a paradoxical shortening of incubation times (*40*), instead of the prolongation predicted from ($Prn\text{-}p^a \times Prn\text{-}p^b$) F1 mice (long incubation times are dominant) (*55–57*). It is unknown whether this paradoxical shortening in transgenic ($Prn\text{-}p^b$) mice results from high levels of PrP^C expression.

Host genes also influence the development of scrapie in sheep. Parry argued that natural scrapie is a genetic disease that could be eradicated by proper breeding protocols (7). He considered its transmission by inoculation of importance primarily for laboratory studies and the communicable infection of little consequence in nature. Other investigators viewed natural scrapie as an infectious disease and argued that a host's genes modulate susceptibility to an endemic infectious agent (*58*). The dominant incubation time gene (*Sip*) for experimental scrapie in Cheviot sheep is thought to be linked to a PrP gene RFLP (*59*), a situation analogous to that for *Prn-i* and *Sinc* in mice. However, the data for genetic linkage in sheep are not convincing and further studies are needed, especially in view of earlier investigations in which susceptibility of sheep to scrapie was thought to be governed by a recessive gene (7). In Suffolk sheep, a polymorphism in PrP was found at codon 171 (Fig. 2B) (*46*); whether this polymorphism segregates with a *Sip* phenotype in Cheviot sheep is uncertain.

Human Familial Prion Diseases

CJD was believed to have a genetic basis when it was recognized that ~10% of CJD cases are familial (*2*). The discovery of the PrP gene (PRNP) in humans (*22, 39*) raised the possibility that mutation might feature in the human prion diseases; a point mutation at PrP codon 102 was found to be genetically linked to GSS syndrome (Fig. 2C) (*3*). The codon 102 mutation has been found in American, British, German, Japanese, Canadian, Israeli, French, and Italian families, as well as in the Austrian family in which GSS was first described; these results suggest that the mutation may have arisen independently multiple times (*60*).

When the codon 102 point mutation was introduced into MoPrP in transgenic mice, spontaneous CNS degeneration occurred, characterized by clinical signs indistinguishable from experimental murine scrapie and neuropathology consisting of widespread spongiform morphology and astrocytic gliosis (*61*). By inference, these results suggest that PrP mutations cause GSS and familial CJD. It is unclear whether low levels of protease-resistant PrP in the brains of transgenic mice with the GSS mutation is PrP^{Sc} or residual PrP^C. Undetectable or low levels of PrP^{Sc} in the brains of these transgenic mice are consistent with the results of transmission experiments that suggest low titers of infectious prions. If brain extracts transmit CNS degeneration to inoculated recipients and the de novo synthesis of prions can be demonstrated by serial passage, then such observations would indicate that prions are devoid of foreign nucleic acid, in accord with studies that use other experimental approaches (*10, 16, 28, 32*).

An insert of 144 bp at codon 53 with six additional octarepeats has been described in individuals with CJD from four families that reside in southern England (Fig. 2C) (*62*); normal individuals have five octarepeats. Genealogical investigations have shown that all four families are related, suggesting that there was a single founder born more than two centuries ago. Seven or nine octarepeats (in addition to the normal five) were found in individuals with CJD, whereas deletion of one octarepeat or four additional octarepeats have been identified in individuals without the neurologic disease (*62*).

For many years the high incidence of CJD among Israeli Jews of Libyan origin was thought to be caused by the consumption of lightly cooked sheep brain or eyeballs (*63*). However, some Libyan and Tunisian Jews in families with CJD have a PrP gene point mutation at codon 200 (*4, 64*). One patient was homozygous for the mutation, but her clinical presentation was similar to that of heterozygotes (*4*); therefore, familial prion diseases are true autosomal dominant disorders like Huntington's disease (*65*). The codon 200 mutation also occurs in Slovaks originating from Orava in north central Czechoslovakia (*60*).

Other point mutations at codons 117, 178, and 198 also segregate with inherited prion diseases (*66*). Some patients once thought to have familial Alzheimer's disease are now known to have prion diseases on the basis of PrP immunostaining of amyloid plaques and PrP gene mutations (*67*). Patients with the codon 198 mutation have numerous neurofibrillary tangles that stain with antibodies to τ and have amyloid plaques (*67*) that are composed largely of a PrP fragment extending from residues 58 to 150 (*68*).

It has been suggested that PrP gene mutations render individuals susceptible to a virus (*36*). The putative scrapie virus is thought to persist in a worldwide reservoir of humans, animals, or insects without causing detectable illness. Yet one in 10^6 individuals develop sporadic CJD and die from a lethal infection, whereas ~100% of people with PrP point mutations or inserts eventually develop neurologic dysfunction. That PrP gene germline mutations in patients and at-risk individuals cause familial prion diseases is supported by the experiments with transgenic mice described above. The transgenic mouse studies also suggest that sporadic CJD arises from the spontaneous conversion of PrP^C to PrP^{CJD} (a component of the prion that causes CJD) due either to a PrP gene somatic mutation or to a rare event involving modification of wild-type PrP^C.

Transgenic Animals and Species Barriers

The species barrier was discovered when scrapie prions were passaged between species; this is a stochastic process characterized by prolonged incubation times (*69*). Prions synthesized de novo reflect the sequence of the host PrP gene and not that of the PrP^{Sc} molecules in the inoculum (*70*). On subsequent passage in a homologous host, the incubation time shortens to a constant length that is observed for all subsequent passages, and transmission becomes a nonstochastic process. The species barrier is of practical importance in assessing the risk for humans of acquiring CJD after consumption of scrapie-infected lamb or BSE-infected beef.

To test the hypothesis that differences in PrP gene sequences might be responsible for the species barrier, we constructed transgenic mice expressing HaPrP (*71, 72*). The PrP genes of Syrian hamsters and mice encode proteins differing at 14 residues. Incubation times in four lines of transgenic mice inoculated with Mo scrapie prions were prolonged, as compared to those observed for nontransgenic, control mice (Fig. 3A). Transgenic mice inoculated with Ha prions showed abbreviated incubation times in a nonstochastic process (Fig. 3B) (*71, 72*). The length of the incubation time after inoculation with Ha prions was inversely proportional to the level of $HaPrP^{C}$ in the brains of the transgenic mice (Fig. 3, B and C) (*71*). $HaPrP^{Sc}$ concentrations in the brains of clinically ill mice were similar in all four transgenic lines inoculated with Ha prions (Fig. 3D). Bioassays of brain extracts from clinically ill transgenic mice inoculated with Mo prions revealed that only Mo prions but no Ha prions were produced (Fig. 3E). Conversely, inoculation of transgenic mice with Ha prions led only to the synthesis of Ha prions (Fig. 3F). Thus, the de novo synthesis of prions in transgenic mice is species specific and reflects the genetic origin of the inoculated prions. Similarly, the neuropathology of transgenic mice is determined by the genetic origin of prion inoculum. Mo prions injected into transgenic mice produced neuropathology characteristic of mice with scrapie. A moderate degree of vacuolation in both the gray and white matter was found, whereas amyloid plaques were rarely detected (Fig. 3G). Inoculation of transgenic mice with Ha prions produced vacuolation of the gray matter, no vacuolation of the white matter, and numerous HaPrP amyloid plaques, characteristic of Syrian hamsters with scrapie (Fig. 3H).

These studies with transgenic mice establish that the PrP gene influences all aspects of scrapie, including the species barrier, the replication of prions, the incubation times, the synthesis of PrP^{Sc}, and the neuropathologic changes.

Prion Multiplication

The mechanism by which prion infectivity increases is unknown. Some investigators believe that a scrapie-specific polynucleotide drives prion replication (*28, 29, 31*). If prions contain a scrapie-specific nucleic acid, then such a molecule would be expected to direct the multiplication of the scrapie agent by a stragety similar to that used by viruses (Fig. 4A). In the absence of any chemical or physical evidence for a scrapie-specific polynucleotide (*16, 28, 32*), it seems reasonable to consider alternative mechanisms that might be responsible for prion biosynthesis. The multiplication of prion infectivity is an exponential process in which the posttranslational conversion of PrP^{C} or a precursor to PrP^{Sc} appears to be obligatory (*49*). A PrP^{Sc} molecule might combine with a PrP^{C} molecule to produce a heterodimer that is subsequently transformed into two PrP^{Sc} molecules (Fig. 4B). In the next cycle, two PrP^{Sc} molecules combine with two PrP^{C} molecules, giving rise to four PrP^{Sc} molecules that combine with four PrP^{C} molecules, creating an

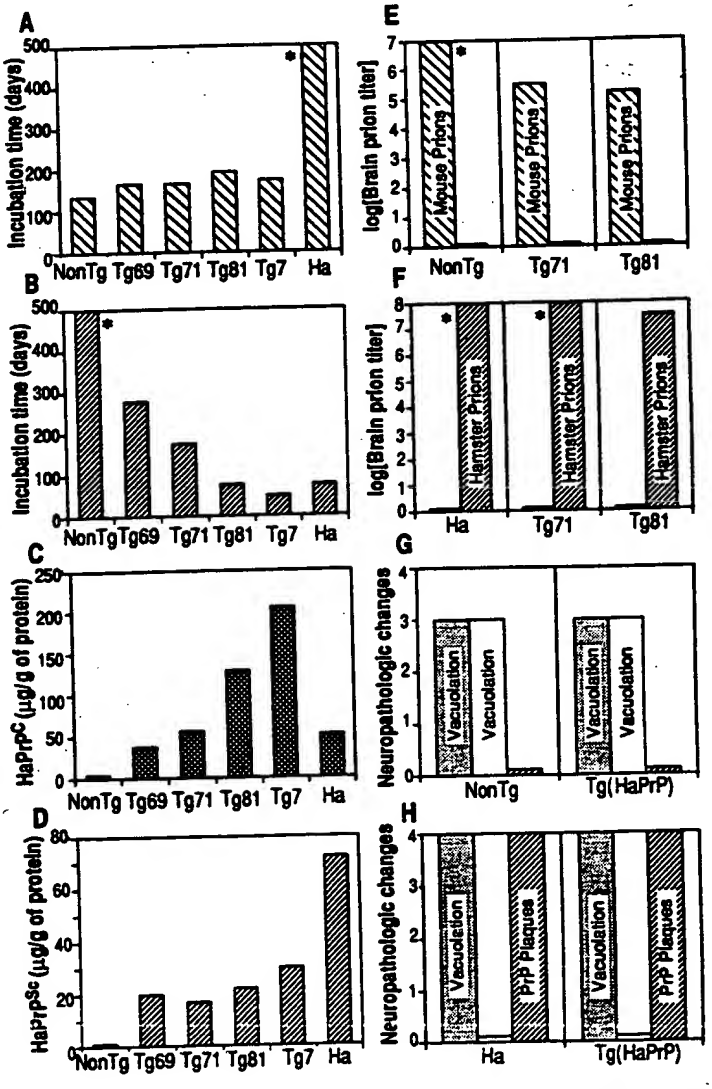

Fig. 3. Transgenic mice expressing Syrian Ha prion protein exhibit species-specific scrapie incubation times, infectious prion synthesis, and neuropathology (*71*). The number of mice used in each trial and the mean ± SEM values can be found in (*71*) for (A) through (F). Asterisks indicate those values that exceed the scales in the *y* axes. (**A**) Scrapie incubation times in nontransgenic mice (NonTg) and four lines of transgenic mice expressing HaPrP and Syrian hamsters inoculated intracerebrally with ~10^6 ID_{50} units of Chandler Mo prions serially passaged in Swiss mice. The four lines of transgenic mice have different numbers of transgene copies: Tg69 and Tg71 mice have 2 to 4 copies of the HaPrP transgene, whereas Tg81 mice have 30 to 50 and Tg7 mice have >60. Incubation times are the number of days from inoculation to onset of neurologic dysfunction. (**B**) Scrapie incubation times in mice and hamsters inoculated with ~10^7 ID_{50} units of Sc237 prions serially passaged in Syrian hamsters and as described in (A). (**C**) Brain $HaPrP^{C}$ in transgenic mice and hamsters. $HaPrP^{C}$ levels were quantitated by an enzyme-linked immunoassay. (**D**) Brain $HaPrP^{Sc}$ in transgenic mice and hamsters. Animals were killed after exhibiting clinical signs of scrapie. $HaPrP^{Sc}$ levels were determined by immunoassay. (**E**) Prion titers in brains of clinically ill animals after inoculation with Mo prions. Brain extracts from NonTg, Tg71, and Tg81 mice were bioassayed for prions in mice (left) and hamsters (right). (**F**) Prion titers in brains of clinically ill animals after inoculation with Ha prions. Brain extracts from Syrian hamsters as well as Tg71 and Tg81 mice were bioassayed for prions in mice (left) and hamsters (right). (**G**) Neuropathology in NonTg mice and Tg(HaPrP) mice with clinical signs of scrapie after inoculation with Mo prions. Vacuolation in gray (left) and white matter (center); PrP amyloid plaques (right). Vacuolation score: 0 = none, 1 = rare, 2 = modest, 3 = moderate, and 4 = intense. PrP amyloid plaque frequency: 0 = none, 1 = rare, 2 = few, 3 = many, and 4 = numerous. (**H**) Neuropathology in Syrian hamsters and transgenic mice inoculated with Ha prions. Degree of vacuolation and frequency of PrP amyloid plaques as in (G).

exponential process. Results from transgenic mice expressing Ha PrP transgenes show that the mice produce only those prions present in the inoculum (Fig. 3, E and F) (*71*). Presumably, PrP^{Sc} in the prion inoculum interacts with the homologous PrP^{C} substrate during replication to produce more of the same prions (Fig. 4C).

In the absence of any candidate posttranslational chemical modifications (*26*) that differentiate PrP^{C} from PrP^{Sc}, we must consider the possibility that conformation distinguishes these isoforms. Various isolates of scrapie prions (*31*) might result from multiple conformers that could act as templates for the folding of de novo synthesized PrP^{Sc} molecules during prion replication (Fig. 4D). Although this proposal is unorthodox, it is consistent with observations from transgenic mice studies that indicate that PrP^{Sc} in the inoculum binds to homologous PrP^{C} or a precursor to form a heterodimeric intermediate in the replication process (*71*). Presumably, "foldases," chaperones, or other macromolecules (*73*) feature in the conversion of the PrP^{C}-PrP^{Sc} heterodimer to PrP^{Sc} molecules. The number of PrP^{Sc} molecules composing a prion particle is unknown, but ionizing radiation studies indicate a target size of 55 kD, suggesting that a PrP^{Sc} dimer or possibly trimer is required for infectivity (*74*).

Two isolates of Ha prions inoculated into transgenic mice and different species of hamsters gave results indicating that the sequence and metabolism of PrP may profoundly influence the isolate phenotype. The Sc237 isolate of Ha prions produced incubation times of 77 ± 1 day (n = 48) in Syrian hamsters, whereas the 139H isolate yielded incubation times of 168 ± 7 day (n = 54) (*31*). $HaPrP^{C}$ expression in Tg(HaPrP)7 mice is approximately fivefold higher than in Syrian hamsters (Fig. 3C). In Tg(HaPrP)7 mice, the Sc237 isolate produced incubation times of 48 ± 1 day (n = 26), whereas 139H gave incubation times of 40 ± 3 day (n = 11) (*75*). One interpretation of these observations is that Sc237 prions have a higher affinity for PrP^{C} than 139H prions that is only apparent at nonsaturating levels of substrate. Increased levels of PrP^{C} substrate in Tg(HaPrP)7 mice might saturate the PrP^{Sc} conversion process, thus resulting in a diminution of the incubation times for both prion isolates and eliminating the differences between them. In Chinese and Armenian hamsters with PrP gene sequences that differ from that of the Syrian at 7 and 8 codons, respectively (*47*), 139H produces incubation times that are either shorter or similar to those observed with Sc237. In this case, the amino acid sequence of PrP may modulate the affinities of PrP^{Sc} in the two isolates for PrP^{C} molecules; indeed, the formation of PrP^{C}-PrP^{Sc} heterodimers may be the rate-limiting step in the prion biosynthesis that determines scrapie incubation times (Fig. 4D).

In humans carrying point mutations or inserts in their PrP genes, mutant PrP^{C} molecules might spontaneously convert into PrP^{Sc} (Fig. 4E). Although the initial stochastic event may be inefficient, once it happens the process would then become autocatalytic. The proposed mechanism explains the existence of individuals harboring germline mutations who do not develop CNS dysfunction for decades and is also consistent with results with transgenic mice that express the GSS mutation and spontaneously develop CNS degeneration (*61*). Whether all GSS and familial CJD cases are attributable to infectious prions or whether some represent inborn errors of PrP metabolism in which neither PrP^{Sc} nor prion infectivity accumulates is unknown.

Conformational changes in allosteric enzymes induced by phosphorylation or the binding of small ligands (*76*) might provide some precedent for the proposed models (Fig. 4, B through E). Consider the possibility that PrP^{Sc} acts as a ligand that induces a conformational change in PrP^{C} to produce a second PrP^{Sc} molecule. Noteworthy are five different crystalline allomorphs of mutant lysozyme from bacteriophage T4 (*77*); these are thought to represent a continuous range of conformations that occur in solution. Rapid- and slow-folding populations of lysozyme have been observed; the latter are presumed to have arisen from *cis-trans* isomerization of peptide bonds preceding proline residues (*78*). Whether *cis-trans* proline isomerization is of significance in the conversion of PrP^{C} or a precursor to PrP^{Sc} is uncertain. Of interest are the folding and assembly of phage tail spike proteins into trimers that resist denaturation and proteolysis, properties remiscent of those exhibited by PrP^{Sc} (*79*). In ciliates, the cytoplasmic inheritance of asymmetrical arrangements of surface structures (*80*) may also provide some insight into the mechanism by which PrP^{C} is converted to PrP^{Sc} during the propagation of distinct scrapie isolates.

Although results with transgenic mice argue for the interaction of PrP^{Sc} with PrP^{C} during scrapie prion multiplication, there are no data to support the proposal that prion multiplication proceeds through a crystallization process involving PrP amyloid formation (*81*). The absence or rarity of amyloid plaques in many prion diseases, as well as the inability to identify any amyloid-like polymers in cultured cells that synthesize prions, does not support this hypothesis (*37, 71*). Purified infectious preparations isolated from scrapie-infected Ha brains contain PrP^{Sc} molecules that exist as amorphous aggregates; only if PrP^{Sc} is exposed to detergents and limited proteolysis does it polymerize into prion rods with the ultrastructural and tinctorial features of amyloid (*37*). Furthermore,

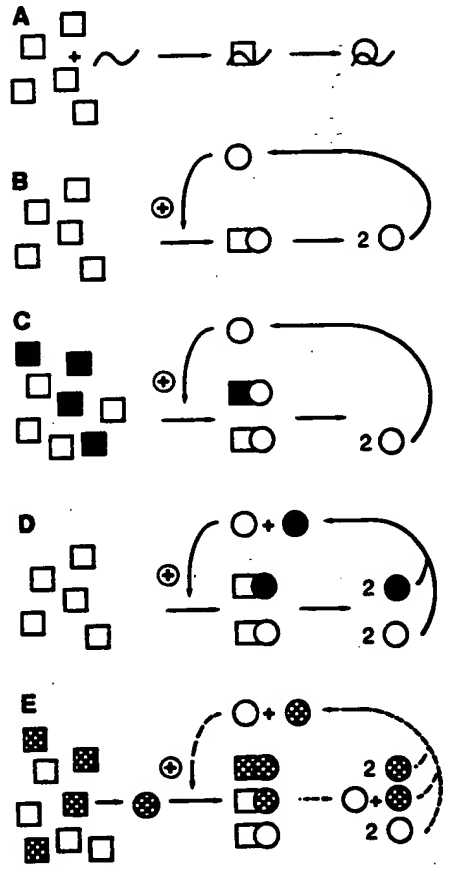

Fig. 4. Some possible mechanisms of prion replication. (**A**) Two-component prion model. Prions contain a putative, as yet unidentified, nucleic acid or other second component (solid, thick wavy line) that binds to PrP^{C} (squares) and stimulates conversion of PrP^{C} or a precursor to PrP^{Sc} (circles). (**B**) One-component prion model—prions devoid of nucleic acid. PrP^{Sc} binds to PrP^{C} forming heterodimers that function as replication intermediates in the synthesis of PrP^{Sc}. Repeated cycles of this process result in an exponential increase in PrP^{Sc}. (**C**) Prion synthesis in transgenic mice (*71*). $HaPrP^{Sc}$ (circles) binds to $HaPrP^{C}$ (white squares), leading to the synthesis of PrP^{Sc}. Binding to $MoPrP^{C}$ (black squares) does not produce PrP^{Sc}. Species barrier for scrapie between mice and hamsters represented by MoPrP^{C}-HaPrP^{Sc} heterodimer. (**D**) Scrapie isolates or strains in hamsters or mice. Multiple PrP^{Sc} conformers (circles) bind to PrP^{C} and constrain the conformational changes that PrP^{C} undergoes during its conversion into PrP^{Sc}. (**E**) Inherited prion diseases in humans and transgenic mice. Mutant PrP^{C} molecules (checkered pattern in squares) might initiate the conversion of PrP^{C} to PrP^{Sc} (or PrP^{CJD}). If infectious prions are produced (dashed lines), then they stimulate the synthesis of more PrP^{CJD} in humans and PrP^{Sc} in experimental animals. Alternatively, prion infectivity is not generated, but the host develops neurologic dysfunction, spongiform degeneration, astrocytic gliosis, and possibly PrP amyloid plaques (*2, 3, 60, 61*).

dispersion of prion rods into liposomes results in a 10- to 100-fold increase in scrapie prion titer; no rods could be identified in these fractions by electron microscopy (*17*).

Future Challenges and New Approaches

Whether prions are composed entirely of PrP^{Sc} molecules or contain a second component needs to be resolved. Determining the crystal structures of PrP^{C} and PrP^{Sc}, as well as the structures of these molecules in solution, is important. Understanding the molecular events that feature in prion replication should help decipher the structural basis for the scrapie isolates or strains that have different incubation times in the same host. Whether distinct conformations of PrP^{Sc} correspond to different prion isolates is unknown. Elucidating the function of PrP^{C} might extend our understanding of the pathogenesis of prion diseases and point to other macromolecules that participate in a variety of human and animal diseases of unknown etiology. Lessons learned from prion diseases may give insights into the etiologies, as well as the pathogenic mechanisms, of such common CNS degenerative disorders as Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease.

The lack of effective therapies for the prion diseases, all of which are fatal, poses a significant challenge. Because the mechanism of prion replication appears unprecedented, it is not surprising that antibacterial, fungal, and viral therapeutics are of little value in the modification of the course of prion diseases. On the other hand, prenatal testing in families with prion diseases does present a method for controlling the genetic spread of these disorders.

Although the results of many studies indicate that prions are a new class of pathogens distinct from both viroids and viruses, it is unknown whether different types of prions exist. Are there prions that contain modified proteins other than PrP^{Sc}? Assessing how widespread prions are in nature and defining their subclasses are subjects for future investigation. Elucidation of the mechanism by which brain cells cease to function and die in prion diseases after a long delay may offer approaches to understanding how neurons develop, mature, and continue to transmit signals for decades.

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Virus Scare Halts Hormone Research

Three deaths attributed to a brain virus have halted distribution of human growth hormone and other products of the pituitary gland

Early in March, officials at the National Institutes of Health (NIH) received a letter from a Stanford University physician that contained some disturbing information. Autopsy reports, the letter said, had confirmed that a young man who died last November had been a victim of Creutzfeldt-Jakob disease, a rare and mysterious viral infection of the brain for which there is no known cure. The source of his infection was believed to be growth hormone, extracted from human pituitary glands, with which he was treated between 1966 and 1976.

The letter sparked a series of events that have brought to a halt the distribution within the United States of growth hormone to treat certain types of dwarfism. It has also brought to an end, for an indefinite period, clinical research on all hormones extracted from pituitary glands, and placed in doubt the future of the National Hormone and Pituitary Program, a 20-year federal program that produces a variety of hormones for research and distributes growth hormone free of charge to patients in the United States.

About the only potential winners in all this are biotechnology companies that are poised to market synthetic versions of growth hormone derived from recombinant DNA techniques. Their products now have no competitors. Genentech of South San Francisco and KabiVitrum, a Swedish company, have been conducting clinical trials of a biosynthetic version of the hormone and Genentech is expected soon to receive approval from the Food and Drug Administration (FDA) to market its product in the United States.

These actions have provoked some controversy. "What we have is panic, what we have is jitter, what we have is supercaution," says Albert Parlow, an endocrinologist at Harbor-UCLA Medical Center who since 1977 has extracted and purified pituitary hormones under contract to NIH. "It is a major tragedy," he says. Erol Caglarcan, a spokesman for Serono Labs, one of two commercial suppliers of natural human growth hormone in the United States, says, "We think the [federal government] acted much too hastily."

Others argue that the government had no choice. When he received the letter from Stanford, Mortimer Lipsett, director of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, which runs the national pituitary hormone program, immediately called a meeting to determine what action to take. It was decided to halt all clinical experiments with pituitary hormones that do not involve therapy. Then in the following few weeks, NIH learned of two more suspected cases of Creutzfeldt-Jakob disease in patients who had received growth hormone in the 1960's and 1970's. One died in February but no autopsy was performed. The second died in April and the diagnosis was tentatively confirmed by autopsy.

Albert Parlow

Research with pituitary hormones could be destroyed.

"That clinched it," says Lipsett. NIH promptly sent out a notice halting distribution through the federal program of new batches of growth hormone. FDA also put pressure on Serono and KabiVitrum, the two commercial suppliers of the hormone in the United States, to halt distribution as well. "I don't think NIH had any choice," says Stanley Prusiner of the University of California at San Francisco who is an expert on the class of viruses that includes Creutzfeldt-Jakob. "These kinds of degenerative diseases in young people are extremely uncommon," he notes. Caglarcan of Serono points out that doubts have been expressed about the diagnoses in the three cases, but Prusiner says that is not at all unusual with this disease. The consensus seems to be that the autopsy reports provide strong evidence in at least those two cases.

All three victims received hormone through the federal program at a time when the preparations were relatively crude. The material is extracted from pituitary glands taken from the brains of donors—usually accident victims—and put through a series of purification procedures. Before 1977, the final product was only between 25 percent and 50 percent pure, the remainder being a variety of unknown proteins. The supposition is that if any of the glands were infected with virus, the purification techniques may not have been rigorous enough to keep it out of the final product.

More recent techniques produce a product that is about 95 percent pure, however. Parlow and Caglarcan argue that there is no evidence that the highly purified versions of the hormone pose any danger of contamination with Creutzfeldt-Jakob virus.

The halt in distribution of the hormones is supposed to be temporary while tests are run to determine what the risks, if any, are. But in fact it could take years before supplies of natural pituitary hormones can be proved safe to everybody's satisfaction. In the meantime, biosynthetic versions of growth hormone are likely to enter the market and distribution of the natural version may never be resumed in the United States. If that were to happen, the National Hormone and Pituitary Program, which currently processes some 50,000 pituitary glands a year could be wound up. This would mean that a variety of pituitary hormones for which there are no alternative sources could remain permanently unavailable for clinical research. "We could be facing the destruction of research with hormones of the pituitary gland," says Parlow.

The immediate problem, however, is that treatment for an estimated 3500 children and adolescents with pituitary disorders who were being supplied with

growth hormone is in jeopardy. Some 2300 were receiving growth hormone through the federal program and the remainder were being treated with commercially produced material at a cost of between $5,000 and $10,000 per year. (Exactly how many are being treated is uncertain. Serono says it is supplying hormone for at least 1500 children, but there may be overlap with those receiving at least part of their supplies through the federal program.) Although physicians are free to use supplies they already have on hand, the halt in distribution of new material will dry up availability of the hormone within 2 months, federal officials believe. The impending unavailability of growth hormone will put intense pressure on FDA to approve Genentech's application. But even if biosynthetic versions are soon available, those who have been receiving hormone free through the federal program will be faced with potentially enormous costs of buying it commercially.

The chief reason why it will take so long to determine the safety of pituitary extracts is that Creutzfeldt-Jakob virus is extremely difficult to detect and work with. It has a long incubation period, which appears to increase as the initial dose is decreased. Thus a very low level of contamination of hormone samples will be very hard to confirm and even more difficult to discount completely.

As a first step, NIH is planning to inject samples of every batch of growth hormone ever distributed by the National Hormone and Pituitary Program—fortunately such samples have been retained—into squirrel monkeys to see whether they come down with Creutzfeldt-Jakob disease. It will take perhaps 18 months to obtain a positive result, but even longer to say with certainty that the animals were not infected.

At the same time, NIH is hoping to do an epidemiological study of those who have received growth hormone through the federal program. Such a study could take at least a year, according to federal officials. Serono has already conducted a study of 300 people in Switzerland who have been treated with its hormone, but found no evidence of Creutzfeldt-Jakob disease, according to Caglarcan.

Finally, some tests may be run using scrapie virus, which produces a disease in sheep similar to Creutzfeldt-Jakob, to get some indication of whether the new purification techniques are effective in screening out these types of viruses. Such a test would involve spiking pituitary glands with scrapie virus, running them through the extraction and purification processes, and testing the product for presence of scrapie virus. The final tests would involve injecting the products into hamsters, which should show symptoms within about 3 months, according to Prusiner.

Similar tests with scrapie virus were in fact conducted in Britain in the late 1970's, and they indicated that no virus was present after the pituitary extracts had been purified. "This is very encouraging, but we don't feel it is conclusive," says Judith Fradkin, head of the endocrinology branch at the arthritis institute. Even if a repeat of the British tests produces similar results, there is some uncertainty about extrapolating the findings to Creutzfeldt-Jakob virus. Prusiner says it would be at best "a good first approximation." According to Fradkin, a meeting will soon be held to design such tests and determine whether they would be worth doing.

Unless biosynthesized growth hormone is approved soon, treatment for some 3500 patients will be in jeopardy.

In the meantime, clinical research with pituitary hormones is on hold. Most of the research involves growth hormone—in fact, all the people being treated through the National Hormone and Pituitary Program were participating in clinical studies. As Salvatore Raiti, the director of the program points out, "We know that human growth hormone works, but we still don't even know the optimal dose." The program was, however, about to make some other pituitary hormones available for clinical research. (All the materials are extracted and purified by Parlow at UCLA. He supplies them to the national program, which is based at the University of Maryland Medical School in Baltimore, and they are distributed from there to researchers. The whole operation is funded by NIH.)

For example, some 7 grams of highly purified prolactin are available for the first time. Prolactin stimulates milk production in lactating women, but it is also produced in substantial quantities by males for purposes that have not been determined. Mark Molitch of Northwestern University Medical School had just begun some initial pharmacology studies with the material, and several investigators were planning experiments, according to Raiti. There are no alternative sources of pure prolactin.

Similarly, the program was about to announce that highly pure thyroid-stimulating hormone (TSH) is ready for clinical investigations. The material should be useful for studying thyroid function and may eventually prove valuable in treating thyroid tumors. An alternative, bovine TSH, is available only in relatively crude extracts and causes an immune reaction, according to Parlow.

The sex hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), have also been prepared individually in pure form. These hormones are also extracted from the urine of postmenopausal women, but this produces a mixture of the two which is unsuitable for some research applications. According to Raiti, these hormones have been unavailable from the program for several years, but sufficient quantities had just been accumulated to make them available again.

Clinical studies with pituitary hormones can only be resumed when it has been determined that there is no danger of viral contamination. The national program's supporters worry, however, that the program itself will be abandoned before that happens.

Concerns about the program's future were, in fact, already being expressed even before the Creutzfeldt-Jakob scare. The problem is that, in distributing growth hormone free of charge, the program has been competing with commercial suppliers. Although this has not presented problems in the past because demand for the hormone has exceeded supplies, once biosynthetic versions come on the market, there is likely to be pressure on the federal government to stop competing with the private sector.

Raiti argues that the program is "an enormous bargain." He points out that it costs just $1.2 million a year and is providing growth hormone to 2300 patients—a cost of about $500 per patient. And that calculation does not even include the benefits from providing other hormones for research. In contrast, treatment with commercially produced growth hormone costs $5,000 to $10,000 a year.

If the production and distribution of growth hormone is halted indefinitely, will it be worth processing 50,000 pituitary glands a year to produce small quantities of the other hormones for research, even if the products are eventually pronounced safe? "I don't know," says Lipsett. The future of the program is now being looked at, he says.

—COLIN NORMAN

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SPECIAL ARTICLE

POTENTIAL EPIDEMIC OF CREUTZFELDT–JAKOB DISEASE FROM HUMAN GROWTH HORMONE THERAPY

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A DECADE after the demonstration that Creutzfeldt–Jakob disease could be accidentally transmitted from one person to another during brain or eye surgery,[1,2] iatrogenic Creutzfeldt–Jakob disease has reappeared as a result of earlier therapy with human growth hormone, with the ominous possibility of a burgeoning epidemic.

Within just a few months, three young adults in the United States have died from the disease, confirmed neuropathologically in two patients and clinically detected but unconfirmed in one[3]; an additional neuropathologically confirmed case has been identified in Great Britain.[4] Salient data on these four cases are summarized in Table 1. (The details of both confirmed American cases[5,6] are reported in this issue of the *Journal*.)

Four other young adult U.S. growth hormone recipients who later died of chronic neurologic diseases that were not clinically characteristic of Creutzfeldt–Jakob disease are also under investigation. Their antemortem diagnoses were postradiation encephalopathy (two patients), atypical motor neuron disease (one), and atypical multiple sclerosis (one).

Creutzfeldt–Jakob disease has a worldwide yearly incidence of about one case per million population in countries where physicians are fully aware of the diagnosis and where it has been vigorously sought.[7,8] However, only 9 previous cases in patients under 30 years of age[9,10] are known among more than 3000 worldwide cases either reported in the literature or referred to us for experimental transmission studies, and 3 of these cases were iatrogenic.[1,2] The age-specific mortality rate for Creutzfeldt–Jakob disease in the population under 40 years of age is only 0.01 case per million (Brown P, et al.: unpublished data). Since approximately 10,000 Americans, all under 40, have received human growth hormone, the expected incidence of Creutzfeldt–Jakob disease in this group is 0.0001 case per year — i.e., the chance of one case occurring in a given year is 1 in 10^4. The chance of three cases occurring in one year is 1 in 10^{12}, and the chance of six cases occurring in one year is 1 in 10^{24}. Thus, the abrupt appearance of at least three cases of the disease in Americans under the age of 40 who had all been treated with growth hormone derived from pools of human pituitary glands obtained at autopsy strongly incriminates Creutzfeldt–Jakob disease–contaminated growth hormone as the cause.

A potential conflict exists between the legitimate interests of patients, parents, and physicians, many of whom are willing to accept a small risk of iatrogenic Creutzfeldt–Jakob disease rather than relinquish the benefit of therapy with human growth hormone; pharmaceutical firms that make the hormone, which wish both to exonerate their products and continue to market them; and public health officials and scientists, who must decide what degree of risk attends treatment with human growth hormone and formulate a policy that minimizes this risk yet does not ignore the hormonal needs of patients.

From the available data, and allowing for the influence of variables that are less well defined, one can estimate the risk of inadvertent contamination of human growth hormone by Creutzfeldt–Jakob disease virus. The U.S. annual mortality rate from all causes during the 1960-1980 period was approximately 0.9 per cent, or, in the population of 250 million, somewhat fewer than 2.5 million deaths each year. Since the annual mortality rate from Creutzfeldt–Jakob disease is approximately 0.7 to 1.0 per million, or, in the U.S. population, somewhat fewer than 250 deaths per year, it follows that roughly 1 in 10,000 deaths in this country is due to this disease. Because lots of pituitaries used in the preparation of human growth hormone have varied from 500 to nearly 20,000 glands, frequent episodes of contamination can be expected to have occurred, unless patients with Creutzfeldt–Jakob disease were systematically excluded as sources of pituitary glands. Such exclusion was unlikely for at least two reasons: (1) patients with chronic neurologic diseases were not banned from collection, and until the mid-1970s diagnostic awareness

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Table 1. Recipients of Human Growth Hormone (HGH) Who Have Died of Creutzfeldt–Jakob Disease (CJD).*

Patient no.	1	2	3	4
Sex/age at death	M/21	M/34	M/23	F/22
Reporting medical center	UCSF/Stanford† (U.S.)	Dallas (U.S.)	Buffalo (U.S.)	London/Winchester/ Southhampton (U.K.)
Cause of pituitary deficiency	Idiopathic	Idiopathic	Idiopathic	Craniopharyngioma‡
HGH therapy				
Begun	1966	1963	1969	1972
Ended	1980	1969	1977	1976
No. of lots	22§	6	15	9
CJD				
Onset	May 1984	Apr. 1984	Sept. 1983	Mar. 1984
Death	Nov. 1984	Feb. 1985	Apr. 1985	Feb. 1985
Interval between HGH and onset of CJD				
Minimum (yr)	4	15	6	8
Maximum (yr)	18	21	13	12
Neuropathological verification	Yes	No¶	Yes	Yes

*Data on U.S. patients were obtained from clinic and hospital records. Patients 1 and 3 are described in detail in this issue of the *Journal*.[5,6]

†University of California, San Francisco.

‡Neurosurgical ablation in 1964.

§Shared eight lots with Patient 3, one additional lot with Patient 2, none with Patient 4.

¶Exhumation performed and neuropathological examination in progress.

of Creutzfeldt–Jakob disease was rather low in most countries and in many U.S. cities; and (2) because of the years-long incubation period of the disease, some virus-infected patients must have died of other causes before typical neurologic illness became apparent, and so they would have been unknowingly included as source material.

Even this could be a conservative estimate, in that a large proportion of total deaths are due to accidents or chronic diseases for which autopsies are done only infrequently, whereas patients dying with "interesting" or unusual diseases of brief duration (i.e., less than one year) come to autopsy comparatively often; thus, Creutzfeldt–Jakob disease could be overrepresented in an autopsy series. On the other hand, the reluctance in recent years to conduct autopsies on patients with this disease, as well as an increasing awareness of the potential for iatrogenic transmission, could have the opposite effect of reducing the chance that a patient with Creutzfeldt–Jakob disease would be included in an autopsy series.

How likely is the pituitary gland to be infectious in a patient with this disease, and how infectious is it? Virus can be experimentally transmitted from brain tissue in nearly 90 per cent of such patients, and has been repeatedly found in cerebrospinal fluid and tissues of the eye[11]; pituitary tissue, although not yet tested in animal-transmission experiments, must be assumed to be infectious, since the adjacent hypothalamic nuclei are extensively involved in the disease process, and since the neurohypophysis, like the retina, is an extension of the brain itself. Virus titers in affected brains have varied between 10^3 and 10^6 LD_{50} per gram of tissue.

How likely is infectious crude pituitary tissue to retain infectivity after processing to human growth hormone? Protocols for older lots are different from those for more recently prepared lots, and vary for frozen and acetone-fixed tissue and to some extent among different manufacturers. Although none of the filtrations, adsorptions, or chemical treatments used in the protocols would by themselves provide adequate inactivation of the virus causing Creutzfeldt–Jakob disease, the cumulative effect of the procedures could be important. In fact, scrapie virus has been experimentally processed according to one protocol for growth hormone production, and had no detectable infectivity ($\leq 10^{0.5}$ LD_{50} per milliliter) in the final product.[12] On the other hand, since Creutzfeldt–Jakob disease virus resists treatment with many chemicals — e.g., acetone, ether, alcohols, iodine, hydrochloric acid, and formaldehyde — and since minimal infectivity has been present in brain suspensions after treatments as vigorous as 15-minute exposures to sodium hypochlorite, sodium hydroxide, or steam autoclaving[13,14] (and Brown P, et al.: unpublished data), it seems prudent to suppose that although nearly total inactivation of virus may occur, no amount of processing can be guaranteed to result in a fully sterile end product.

In consideration of these variables, the most reasonable approach is to estimate the risk of growth hormone contamination with a "worst case–best case" analysis. The worst case, represented by lot sizes of 20,000 glands, each containing 2 glands with high titers of Creutzfeldt–Jakob disease virus and little or no inactivation by processing to growth hormone, would give lots containing titers of 2×10^6 LD_{50} (10^6 in each of the two diseased glands), diluted by the presence of 2×10^4 total glands. The lot would therefore have a virus titer of 10^2 LD_{50} per gram of tissue, each gram yielding approximately 10 mg of human growth hormone (5 to 10 therapeutic doses, depending on the purity of the preparation).

In the best case, a maximum of 1 in 20 lots of 500 pituitaries would contain a single affected gland, having a crude tissue virus titer of 10^3 LD_{50} or less and undergoing further titer reduction during processing to only a single infectious unit. This infectious unit would occur at random in a single therapeutic dose made from the contaminated lot, with most lots remaining uncontaminated. Since therapy usually consists of intramuscular injections two or three times a week with successive lots of growth hormone over a period of several years, and since intramuscular administration of Creutzfeldt–Jakob disease virus has

been shown to cause disease in primates,[15] it is likely that even under the best conditions, at least an occasional patient would receive a contaminated and potentially disease-producing dose of human growth hormone during the course of therapy.

From numerous experimental transmission studies in which multiple animals have received aliquots of a single brain-tissue suspension infected with Creutzfeldt–Jakob disease, it is known that after incubation periods of several years (depending on the species), clinical onsets tend to occur in a "burst" at the center of a narrow bell-shaped curve, with a few shorter or very much longer peripheral points. The recent cluster of cases of Creutzfeldt–Jakob disease following therapy with human growth hormone could thus represent the initial short-incubation-period points in what will soon become an epidemic of iatrogenic Creutzfeldt–Jakob disease (assuming the worst case); or they may represent the burst itself (assuming the best case), in which event few if any additional patients will be identified.

Epidemiologic studies already in progress will eventually determine whether other recipients of human growth hormone have been or will be affected by Creutzfeldt–Jakob disease, by identifying all patients under 40 who have died with the disease in the United States during the past 10 years and by identifying and following all recipients of the hormone.

The three U.S. patients had been exposed to a total of 33 different lots of human growth hormone during therapy; nine of these lots were shared by Patients 1 and 3, and one other lot was shared by Patients 1 and 2, but no lot was shared by all three patients. Aliquots of the 26 available lots to which these patients were exposed (including all the shared lots) have already been inoculated into chimpanzees and squirrel monkeys (19 of the 22 lots given to Patient 1, 2 of the 6 lots given to Patient 2, and all 15 lots given to Patient 3). Fifty-three additional lots dating as far back as the mid-1960s, not received by these patients, have also been inoculated into squirrel monkeys. Arrangements are under way for similar inoculation experiments with all the available lots received by the British patient, none of which overlapped with any lots given to the U.S. patients. Positive results will require minimum incubation periods of one year in chimpanzees and two years in squirrel monkeys, with the possibility of incubation periods of several years in the event of very low infectivity levels.

Immunoblot and electron microscopical analysis of purified frozen brain tissue from Patient 3 has already confirmed the diagnosis of Creutzfeldt–Jakob disease by the finding of scrapie-associated fibrils and the 27-kilodalton marker protein specifically associated with the subacute spongiform virus encephalopathies.[6] Similar analysis of brain tissue from the growth hormone recipient with a provisional diagnosis of amyotrophic lateral sclerosis has failed to detect either scrapie-associated fibrils or marker protein, confirming the clinical impression that this patient did not have Creutzfeldt–Jakob disease (Brown P, et al.: unpublished data). All available older lots and selected newer lots of human growth hormone administered under National Pituitary and Hormone Program protocols are currently being tested for the marker protein by immunoblot analysis. Although the assay is much less sensitive than animal-transmission experiments, it has the enormous advantage of giving immediate results. Serum samples from patients who have received human growth hormone are being tested for the presence of antibody to this protein, since these patients may have been immunized to any contaminating Creutzfeldt–Jakob disease protein during the course of their multiple intramuscular growth hormone inoculations.

Experimental assessment of the level of infectivity likely to be present in lots of human growth hormone already produced is also in progress. The manufacturing protocols used to produce the hormone are being duplicated with the inclusion of known amounts of Creutzfeldt–Jakob disease virus, and the end product is being inoculated into thousands of laboratory animals. Growth hormone contaminated with Creutzfeldt–Jakob disease, with the addition of ultrafiltration and sodium hydroxide exposure steps, is also being inoculated in an effort to develop a product that, if needed, would be safe for future use. Finally, human growth hormone that is genetically engineered in bacteria is being released to replace the natural product in patients in the high-risk group with panhypopituitarism and hypoglycemia, and intensive further work is under way to produce a fully synthetic duplicate of the hormone that does not induce autoantibodies in patients and will make it unnecessary to derive the hormone from human beings.

We are once again dramatically reminded that human tissues are a source of infectious disease, and that any therapeutic transfer of tissue from one person to another carries an unavoidable risk of transferring the infection. In this context, we must continue to worry about such products as follicle-stimulating hormone, luteinizing hormone, prolactin, and human interferon, as well as skin, bone, bone marrow, dura mater, blood vessel, and nerve grafts and organ transplantation.

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MEDICAL INTELLIGENCE

CREUTZFELDT–JAKOB DISEASE IN A YOUNG ADULT WITH IDIOPATHIC HYPOPITUITARISM

Possible Relation to the Administration of Cadaveric Human Growth Hormone

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CREUTZFELDT–JAKOB disease, a transmissible subacute degeneration of the central nervous system, is generally considered a disease of older adults.[1] We recently evaluated a 20-year-old man with idiopathic hypopituitarism and insulin-dependent diabetes mellitus in whom Creutzfeldt–Jakob disease developed. Since early childhood he had received cadaveric human growth hormone, as well as insulin, thyroid hormone, and more recently testosterone.

CASE REPORT

The patient, a 20-year-old man, was referred to the University of California, San Francisco, for evaluation of progressive gait instability. His history was complicated by multiple deficiencies of pituitary hormones from infancy, as well as insulin-dependent diabetes mellitus. Hypopituitarism was suggested by severe growth retardation, hypoglycemia, and insulin hypersensitivity. A diagnosis of growth hormone deficiency was established when the patient was three years old, after L-arginine infusion and insulin-induced hypoglycemia failed to cause a rise in the concentration of serum growth hormone (>3 ng per milliliter).[2] He was started (in September 1966) on daily 1-mg intramuscular injections of purified human growth hormone (Wilhelmi preparation). Within 15 months after the initiation of this treatment, the patient grew by 23 cm and gained more than 7 kg.[2] He received growth hormone for 13 years, after which it was discontinued (in April 1980). He also received thyroid hormone replacement from the age of 15 months and daily insulin injections from the age of 29 months. Control of diabetes was maintained with ultralente insulin and regular insulin. When the patient was 19 years old, he was given intramuscular injections of testosterone enanthate (200 mg each month) for a period of four months for the treatment of hypogonadotropic hypogonadism.

Three months before referral to the University of California, San Francisco (in May 1984), the 20-year-old patient was noted to have an unsteady gait and, shortly thereafter, slurred speech. Neurologic evaluation one month later was notable for truncal and limb ataxia. A computerized tomographic (CT) brain scan, electroencephalogram, and electronystagmogram were normal. The results of serum chemistry studies, antinuclear-antibody assays, and assays for heavy metals in the blood and urine were normal, as was the cerebrospinal fluid. The severity of the ataxia increased, and one month before the patient was admitted, magnetic-resonance imaging of the head was performed, and the resulting scan interpreted as normal.

On admission, the young man was thin but well developed; he weighed 45.9 kg and was 163.5 cm tall. He appeared apathetic and had mild drooling, and though he was oriented and responsive, his mentation was impaired. His speech was severely dysarthric. Examination of the cranial nerves was notable for impairment of the upward gaze and bilateral horizontal nystagmus. There was mild facial diplegia. The gag reflex was exaggerated, and the tongue movements were slow. When standing, the patient was stooped and had titubation. His gait was broad-based and exceedingly unsteady. There was mild symmetric distal atrophy of the muscles; the tone was diffusely increased and had a rigid quality, with cogwheeling. The patient's strength was mildly diminished, and deep-tendon reflexes were absent. The plantar responses were flexor. The patient had marked truncal and limb ataxia, and repetitive movements were performed slowly and laboriously. Scattered myoclonus was apparent, and there was an exaggerated startle response. The patient responded normally to touch and pain, but his perception of position sense and vibration was impaired in the lower extremities. The general physical examination was normal.

The results of serum chemistry studies, including measurements of ceruloplasmin and serum thyroxine while the patient was receiving thyroxine replacement, were normal. Serologic studies were negative. The cerebrospinal fluid was clear, without cells, and the total protein and glucose levels were 43 mg per deciliter and 145 mg per deciliter (8.05 mmol per liter), respectively. There was no oligoclonal banding. A CT brain scan (GE 8800) with contrast was normal. Brain-stem and visual evoked responses were normal. Motor-nerve conduction was mildly delayed, in a manner consistent with chronic diabetes mellitus. An electroencephalogram showed frequent paroxysmal, synchronous bursts of bilateral high-voltage delta activity.

The patient's condition continued to deteriorate; he became bedridden with increasing dementia, rigidity, and myoclonus. He died, six months after the onset of symptoms, at another hospital. Because of the unusual nature of his disease process, special arrangements were made to obtain his brain for neuropathological examination at the University of California, San Francisco.

The formalin-fixed brain weighed 1215 g. Histologic sections revealed spongiosis throughout the cerebral cortex, the basal ganglia, and the molecular layer of the cerebellar cortex (Fig. 1). The spon-

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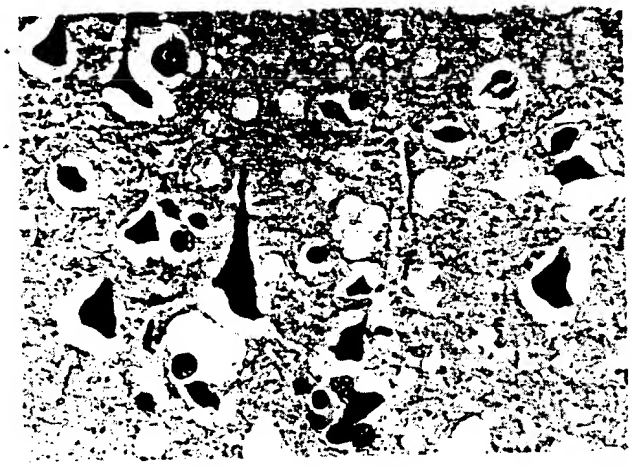

Figure 1. Spongiform Change in the Neuropil between Pyramidal Cells of the Cingulate Gyrus (Hematoxylin–Eosin, ×290).

giosis consisted of small vacuoles located in the neuropil between nerve cell bodies. Loss of nerve cells did not appear to be marked, and little or no reactive gliosis was present. The spongiosis was not found in the hypothalamus, Ammon's horn, brain stem, or spinal cord. These histologic features are identical to those of the "spongiform change" pattern of Creutzfeldt–Jakob disease described by Masters and Richardson.[3] No abnormalities were noted on routine stained sections of the pituitary gland. Immunohistochemical studies showed normal labeling for ACTH and thyroid-stimulating hormone. Staining for cells containing human growth hormone showed that the hormone was present, but in only a small proportion of cells.

Discussion

In this 20-year-old man with idiopathic hypopituitarism and insulin-dependent diabetes mellitus from infancy, an ataxic gait developed, followed shortly thereafter by dysarthria. Within three months the patient had a pancerebellar syndrome, extrapyramidal signs, myoclonus, and dementia that progressed relentlessly until his death. The electroencephalogram showed paroxysmal, synchronous bursts of high-voltage delta activity. This constellation of findings is consistent with a clinical diagnosis of Creutzfeldt–Jakob disease,[4,5] but since the disease usually affects older adults, the patient's age made this diagnosis seem unlikely.

The neuropathological findings were typical of the transmissible spongiform encephalopathies in human beings and animals.[3,6,7] Such spongiform changes are commonly present in patients with Creutzfeldt–Jakob disease who die shortly after the onset of symptoms, and with increasing duration of the disease process, the spongiform change diminishes and gliosis becomes more apparent. The absence of gliosis, however, is not inconsistent with Creutzfeldt–Jakob disease.[3,6,7]

The incidence of Creutzfeldt–Jakob disease has been estimated at one case per million per year.[1] The age at the onset of symptoms in spontaneous cases has ranged from 20 to 79 years, with a peak incidence between 50 and 75.[1,5,8,9] Only rarely have there been spontaneous cases before the age of 30 years.[9-11] Person-to-person transmission was first reported in 1974 as a consequence of corneal transplantation[12]; iatrogenic surgical transmission has occurred at least three times.[1] The disease developed in two patients, 17 and 23 years of age, one to two years after the stereotactic placement of depth electrodes that had earlier been contaminated during use in a patient with Creutzfeldt–Jakob disease.[13] The incubation period for the disease is prolonged — in excess of three years in laboratory animals with direct intracerebral inoculation.[1] In the light of these observations, Gajdusek and associates have recommended procedures for the handling of patient materials and the decontamination of surgical instruments to reduce the risk of transmission.[14,15] The infectious agent involved in the transmissible spongiform encephalopathies appears to have a unique protein as its major component.[16-18] Recently, this protein has been isolated from the brains of patients with Creutzfeldt–Jakob disease.[17]

Retrospective studies indicate that affected patients had significantly more injuries or surgical operations than did controls.[19,20] Our patient did not have a history of serious physical trauma, surgical procedures, or affected relatives; however, for 13 years he had received daily injections of purified growth hormone prepared from human pituitary glands obtained at autopsy.

One possibility that we considered in seeking sources of exposure to the infectious agent of Creutzfeldt–Jakob disease was that one or more of the lots of the purified native growth hormone that our patient had received had been contaminated. Since 1956, human growth hormone has been purified from acetone-dried and frozen pituitary glands obtained at autopsy.[21,22] Early preparations of clinical-grade human growth hormone often contained polymers of growth hormone and marked contamination with other proteins.[23,24] Recent methods of isolation and purification have considerably improved the quality of human growth hormone for use in human beings.[23,24] Human pituitary glands are collected at postmortem examination from patients who are free of systemic infections but not necessarily of degenerative neurologic diseases. It is possible that pituitary glands, including fragments of hypothalamus, from patients who died of Creutzfeldt–Jakob disease could have been included in a large pool of glands (usually about 15,000 glands to a pool) subjected to extraction for human growth hormone. It is not known whether the infectious agent for Creutzfeldt–Jakob disease can be isolated from the pituitary gland. Histologically, our patient's pituitary gland was normal except for decreased immunohistochemical staining for growth hormone.

After the findings in this patient had been reviewed, Dr. Raymond Hintz of Palo Alto, California, the patient's endocrinologist, was informed of the diagnosis. He then notified the directors of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, the National Hormone and Pituitary Program, and the Food and Drug Administration. Since the pos-

sible association of Creutzfeldt–Jakob disease and the administration of human growth hormone was discovered in our patient, two other recipients of long-term therapy with human growth hormone for the treatment of hypopituitarism were found to have died very recently of a degenerative neurologic disease consistent with Creutzfeldt–Jakob disease.[25] All these patients had begun receiving treatment with growth hormone before 1970.[25] In the light of these three cases, the National Hormone and Pituitary Program has temporarily discontinued further distribution of human growth hormone. This matter is being vigorously pursued to clarify the importance and implications of the association between Creutzfeldt–Jakob disease and the administration of native human growth hormone for the treatment of growth hormone deficiency.

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CLINICAL AND PATHOLOGICAL FEATURES AND LABORATORY CONFIRMATION OF CREUTZFELDT–JAKOB DISEASE IN A RECIPIENT OF PITUITARY-DERIVED HUMAN GROWTH HORMONE

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A COMPANION paper in this issue[1] reports on the apparent iatrogenic transmission of Creutzfeldt–Jakob disease to a young adult who had been a recipient of human pituitary growth hormone. This report describes the medical history of another such patient, a young man from Buffalo, and presents data on the clinical, pathological, and laboratory confirmation of Creutzfeldt–Jakob disease by isolation and identification of scrapie-associated fibrils with immunoelectron microscopy using rabbit antibodies to scrapie-associated fibrils, and by identification of the scrapie-associated fibril protein PrP_{27-30} with Western immunoblots using fresh-frozen brain taken at autopsy of the patient. Attempts to isolate scrapie-associated fibrils and the associated PrP_{27-30} protein from formalin-fixed brain tissues were unsuccessful.

CASE REPORT

Clinical Summary

The patient was a 23-year-old man who died after a progressive neurologic disease that lasted for 1½ years.

He first came to medical attention at the age of seven years because he was much shorter than his fraternal twin brother. At that time he was otherwise healthy, and his physical examination was normal except for markedly short stature (height age, 3½ years; bone age, 3 years). His body proportions were normal. Pituitary evaluation revealed growth hormone levels below 1 ng per milliliter after provocative tests using arginine and insulin-induced hypoglycemia. Injections of human growth hormone extract were begun in June 1969, and the patient was maintained on standard doses until October 1977, when he was 15 years old. He also received a few short courses of fluoxymesterone and oxandralone concurrently with growth hormone to augment linear growth and to increase genital size. He responded well to therapy and grew to 166.4 cm.

At 16 years, 10 months, of age, the patient still had abnormally small genitalia, no axillary or facial hair, and only scanty hair on the extremities. The plasma testosterone level was 103 ng per deciliter, luteinizing hormone was undetectable (<1.2 mIU per milliliter), and peak serum levels of follicle-stimulating hormone were 4.2 mIU per milliliter. After infusions of luteinizing hormone–releasing hormone he had no detectable luteinizing hormone, and the response of follicle-stimulating hormone was very poor (peak, 6 mIU per milliliter), confirming gonadotropin deficiency. He was treated with testosterone enanthate, 200 mg every three weeks.

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In 1979 the patient completed high school and entered the U.S. Navy, where he remained for four years. He was discharged and returned home in the fall of 1983. At that time he was first observed to have symptoms and signs of neurologic disease, including a feeling of weakness, mild incoordination while walking, and a tendency to veer to the right. His family noted a marked change in his personality; he had previously been enthusiastic and conscientious, and was now apathetic and careless.

He first entered the hospital for neurologic evaluation in January 1984. Examination revealed that he had an ataxic gait, impairments on finger-to-nose and heel-to-shin tests, and nystagmus on lateral gaze. Laboratory tests, including analysis of cerebrospinal fluid (normal pressure; no cells; total protein, 38 mg per deciliter; and unremarkable findings on protein electrophoresis), measurement of serum and urinary copper and ceruloplasmin, a test for antinuclear antibodies, measurement of the erythrocyte sedimentation rate, routine blood counts, measurement of urinary porphobilinogen, thyroid-function tests, visual and auditory evoked potentials, electroencephalography, electromyography, two computed tomograms of the head, and a nuclear magnetic resonance scan of the brain, all gave normal results. The patient received a 10-day course of ACTH, without any improvement in his clinical signs. The hospitalization lasted for 39 days, during which the patient's balance and gait deteriorated, and he became unable to walk or sit without help.

He was sent home, but was readmitted to the hospital in March 1984 with increasing dementia, head tremor, dysphagia, and myoclonus of the upper extremities. Diagnostic studies, including computed tomograms of the pelvis, abdomen, and chest; upper gastrointestinal tract roentgenograms and a barium enema; a gallium scan; a liver and spleen scan; and an intravenous pyelogram, all gave normal results and revealed no occult malignant tumor. The patient was discharged from the hospital with a tentative diagnosis of Creutzfeldt–Jakob disease and entered a nursing home. There he became totally bedridden, mute, and unresponsive, requiring tube feedings. He died in April 1985.

The patient had received injections of human growth hormone from at least 15 lots over a period of eight years, four months; the therapy had begun 13 years before the onset of neurologic disease, and the last dose had been administered six years before the onset.

Pathological Findings

The autopsy was limited to an examination of the brain and spinal cord. Gross examination of the central nervous system was carried out after fixation in 10 per cent formaldehyde for 13 days. The dura and leptomeninges were unremarkable. The blood vessels comprising the circle of Willis were normal in their anatomical distribution and were free of atherosclerosis. Both cerebral hemispheres had severe generalized atrophy of the cortex with narrow gyri and wide sulci. Diffuse atrophy of the cerebellar cortex was reflected in marked narrowing of the cerebellar folia.

Multiple coronal sections of the cerebral hemispheres showed moderately severe dilatation of the lateral and third ventricles. The cerebral cortical mantle was reduced in width except in the hippocampal regions, which were normal in configuration and size. The subcortical white matter was of normal color and appearance. The caudate nuclei, putamina, and thalami were shrunken. Horizontal sections of the brain stem revealed generalized reduction in the caliber of the midbrain, pons, and medulla, without focal lesions. In horizontal sections of the cerebellum, there was marked narrowing of the folia and widening of the sulci (Fig. 1). The deep cerebellar white matter was unremarkable. External examination of the spinal cord disclosed no abnormalities. Serial sections revealed slight discoloration of the lateral columns.

Sections of the brain and spinal cord were embedded in paraffin and stained with hematoxylin and eosin, luxol fast blue, Congo red, periodic acid–Schiff, and Bodian's method. A marked loss of neurons was observed in the cerebral cortex (except for the hippocampi), basal ganglia, thalamus, and cerebellar cortex. Little or no loss of neurons was detected in the hippocampal region, the tegmental portions of the brain stem, and the posterior and anterior horns of the spinal cord. Neuronal loss was accompanied by striking gliosis characterized by proliferation of astrocytes, including gemistocytes and rod-shaped microglia. Focal areas of spongiform change

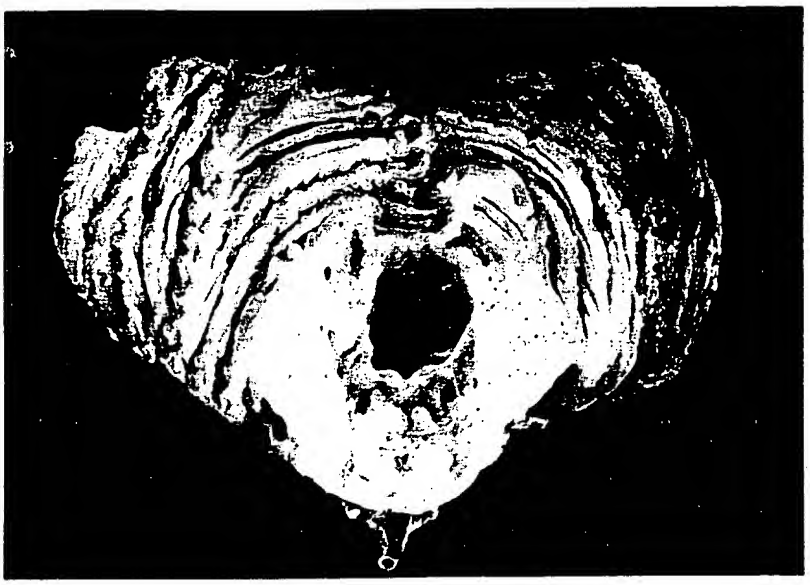

Figure 1. Horizontal Section of Cerebellum and Pons, Illustrating Diffuse Cerebellar Cortical Atrophy.

were present in the frontal, parietal, and occipital areas of the cerebral cortex (Fig. 2). Spongy change was primarily located in the superficial portions of the cortex. In the cerebellum, a marked loss of cells within the internal granular layer had occurred, with paradoxical sparing of the Purkinje cell layer. A patchy loss of Purkinje cells was associated with proliferation of Bergmann's glia (Fig. 3). A mild to moderate loss of neurons had occurred in the dentate nucleus, whereas the neurons of the inferior olivary nuclei were minimally reduced in number. The subcortical white matter of the cerebrum was somewhat pale, indicating a mild loss of myelinated axons. Mild reactive gliosis was seen throughout the subcortical white matter. A mild to moderate loss of myelinated structures was evident in the cerebral peduncles, basis pontis, pyramids of the medulla, and lateral columns of the spinal cord. Reduced myelin staining in luxol fast blue preparations was also noted in the region of the uncrossed corticospinal tracts within the anterior columns of the spinal cord, in the spinocerebellar tracts, and the decussation of the superior cerebellar peduncle in the caudal midbrain.

Methods

Preparation of Scrapie-Associated Fibrils and Protein (PrP_{27-30})

Scrapie-associated fibrils and PrP_{27-30} were extracted from a piece of frontal cortex obtained at autopsy with the method of Merz et al.[2] and Prusiner,[3] as modified by Hilmert and Diringer.[4] Briefly, 1.7 g of frozen brain was homogenized in 25 ml of phosphate-buffered saline (PBS) containing 10 μg of proteinase K per milliliter, using an Ultra-Turax homogenizer (Jahnke and Kunkel IKA-Werk). The homogenate was centrifuged for 10 minutes at 500×g. The supernatant fluid was withdrawn and saved; the pellet was resuspended in 25 ml of fresh PBS containing 10 μg of proteinase K per milliliter and was sonicated and centrifuged as before. The two supernatants were combined and centrifuged for 40 minutes at 22,000×g, after which the supernatant fluid was discarded. The pellet was resuspended in 10 ml of PBS containing 1 per cent sarcosyl and 10 per cent sodium chloride, using sonication, and was then centrifuged for 2.5 hours at 215,000×g in a Beckman 50.2T rotor. The resultant pellet was dissolved with overnight stirring at 37°C in 2 ml of PBS containing 1 per cent sarcosyl and 10 per cent sodium chloride. The next day the suspension was centrifuged for 15 minutes at 7000×g, and the resultant pellet was resuspended in PBS containing 1 per cent sarcosyl, 5 μg of proteinase K per milliliter, and 10 per cent sodium chloride and was incubated for two hours at 37°C. After recentrifugation at 7000×g for 15 minutes the pellets were extracted twice with 0.1 per cent sarcosyl in water for one hour at 37°C and finally resuspended in 400 μl of the 1 per cent sarcosyl–water solution. The final suspension of scrapie-associated fibrils contained 20 μg of protein as determined by the method of Lowry et al.[5]

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis

Scrapie-associated fibrils prepared from the patient's brain and from hamster brains that were experimentally infected with the 263K strain of scrapie were suspended in electrophoresis sample buffer (62.5 mM TRIS hexachlorophene, pH 6.8, 1 per cent mercaptoethanol, and 1 per cent sodium dodecyl sulfate) and were denatured by boiling for two minutes. Proteins were subjected to electrophoresis through 10 per cent polyacrylamide gels as described by Laemmli[6] and were transferred electrophoretically to nitrocellulose membranes by the method of Towbin et al.[7] After transfer the electrophoretic blots were incubated with 5 per cent normal goat serum in phosphate-buffered saline for two hours at 25°C. After washing with PBS the blots were treated with a 1:200 dilution of rabbit anti-scrapie PrP_{27-30} antiserum prepared in PBS containing 5 per cent normal goat serum and incubated for six hours at room temperature. After washing with PBS containing 0.05 per cent Tween 20, the blots were overlaid with biotinylated goat antirabbit IgG antibody (Vector Laboratories, Burlingame, Calif.) for 30 minutes at 25°C, treated with avidin-biotinylated horseradish peroxidase, and washed with PBS, and the horseradish peroxidase reaction was initiated by the addition of 90 mg of 4-chloro-1-naphthol, 60 μl of 30 per cent hydrogen peroxide, and 30 ml of methanol in 150 ml of PBS.

Electron Microscopy and Immunoelectron Microscopy

Small aliquots of scrapie-associated fibril preparations from the patient's brain and aliquots of control hamster brains infected with the 263K strain of scrapie and of normal hamster brains were loaded on carbon-coated 400-mesh grids after glow discharge; the grids were then subjected to negative staining with a 2 per cent aqueous solution of uranyl acetate after brief washing with deionized water.

Immunoelectron microscopy was performed according to the method of DeArmond et al.[8] Samples of scrapie-associated fibrils were applied to carbon-coated grids, which were washed in PBS for 60 minutes. Grids were then transferred to PBS containing 1 per

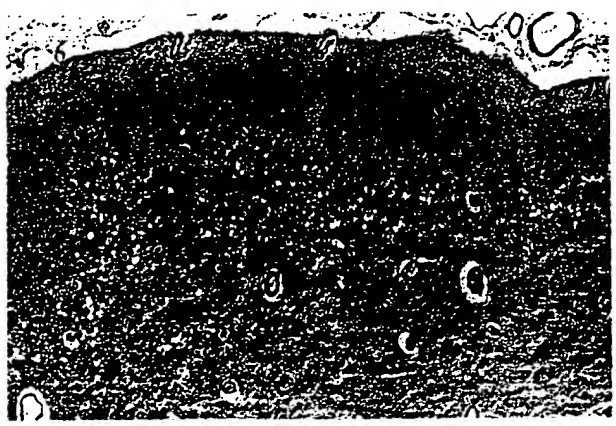

Figure 2. Widespread Spongiform Change in a Portion of Frontal Cortex (Hematoxylin and Eosin Stain, ×36).

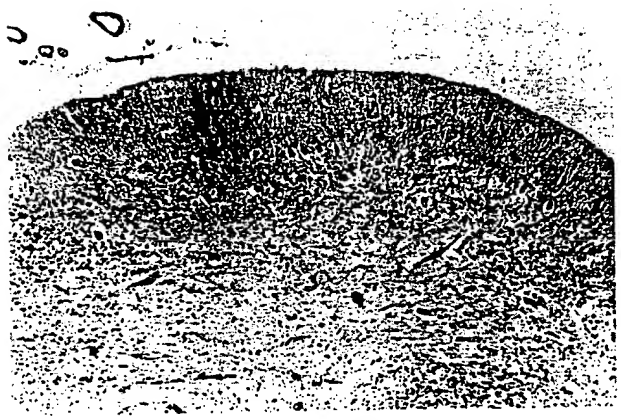

Figure 3. Marked Loss of Internal Granular Cells, with Relative Sparing of Purkinje Cells, in the Cerebellar Cortex (Hematoxylin and Eosin Stain, ×46).

cent bovine serum albumin for 30 to 60 minutes to reduce nonspecific staining, and rabbit antiserum raised to scrapie-infected hamster-brain scrapie-associated fibrils and diluted 1:100 in PBS containing 0.5 per cent bovine serum albumin was applied for 60 minutes. The grids were washed six times in 0.5 per cent bovine serum albumin and PBS and transferred to a solution of sheep antirabbit IgG and colloidal gold diluted 1:10 in 0.5 per cent bovine serum albumin and PBS and 0.02 per cent polyethylene glycol 20,000. After 20 minutes, the grids were washed six times with 0.02 per cent polyethylene glycol 20,000 and once with deionized water, then negatively stained with uranyl acetate as above.

Examination of all grids was carried out using a Phillips EM-201 electron microscope at 80 kV.

Results

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Western Immunoblotting

Electrophoresis followed by silver staining revealed a diffuse band at 27 to 30 kilodaltons in scrapie-associated fibril preparations derived from 1.7 g of frozen frontal cortex of the patient's brain and from scrapie-infected hamster brains that had been lysed in 1 per cent sarcosyl and digested with proteinase K (Fig. 4A). Similar bands were not elicited in preparations derived from 25 g of the patient's brain that had been fixed in 10 per cent formalin.

In further studies of the PrP_{27-30} protein in the scrapie-associated fibrils isolated from the patient's brain, we performed Western immunoblotting with an anti-scrapie (strain ME-7) antibody. As shown in Figure 4B the antibody clearly stained for the 27- to 30-kilodalton protein bands in both the patient's isolate and the scrapie-infected hamster-brain fibril preparation. Similar bands were not detected in immunoblots of material derived from formalin-fixed brain.

Electron and Immunoelectron Microscopy

Negative staining of scrapie-associated fibril preparations from frozen frontal cortex of the patient's brain revealed fibril structures, approximately 20 nm in width, that were twisted periodically into a double-helix-like configuration with sharply cut ends (Fig. 5A). In some areas of the grids large aggregates of these structures were observed. They were indistinguishable from scrapie-associated fibrils extracted from scrapie-infected hamster brains, as shown in Figure 5B.

To confirm the observation that the fibrils isolated from the patient's brain represented the same structures found only in kuru, Creutzfeldt–Jakob disease, and scrapie-infected tissues, we compared the immunoreactivity of colloidal gold particles coated with rabbit antiserum directed against scrapie-infected hamster scrapie-associated fibrils on scrapie-associated fibrils from the patient and from scrapie-infected hamsters. As shown in Figure 6, the antibodies to scrapie-associated fibrils resulted in adherence of colloidal gold particles on fibrils from both the patient and the infected hamsters. There was no binding of the antibody-coated colloidal gold to other structures in the preparation (e.g., collagen fibers), nor did normal control rabbit serum bind to any structures.

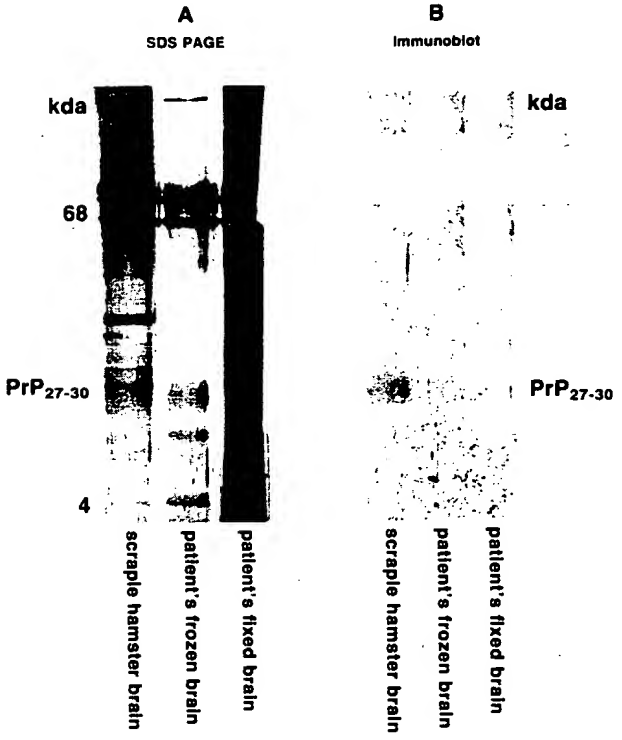

Figure 4. Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS PAGE) and Immunoblot Analysis of Scrapie-Associated Fibril Protein (PrP_{27-30}).

In Panel A, PrP_{27-30} is separated by SDS PAGE (10 per cent) and visualized by silver staining. The sizes of marker proteins are given in kilodaltons (kda). The scrapie-associated fibril protein in the left lane is from scrapie (strain 263K)-infected hamster brain, and that in the center lane is from the patient's frozen brain. The right lane shows the absence of the protein in preparations of the patient's formalin-fixed brain.

Panel B shows immunoblots of the PrP protein in Panel A, stained with a 1:200 dilution of rabbit antibody against scrapie-associated fibrils from scrapie (strain ME-7)-infected mice with biotinylated horseradish peroxidase–conjugated goat antirabbit serum. The PrP_{27-30} in the left lane is from a scrapie-infected hamster brain, and that in the center lane is from the patient's frozen brain. The right lane shows the absence of the protein in a preparation from the patient's formalin-fixed brain.

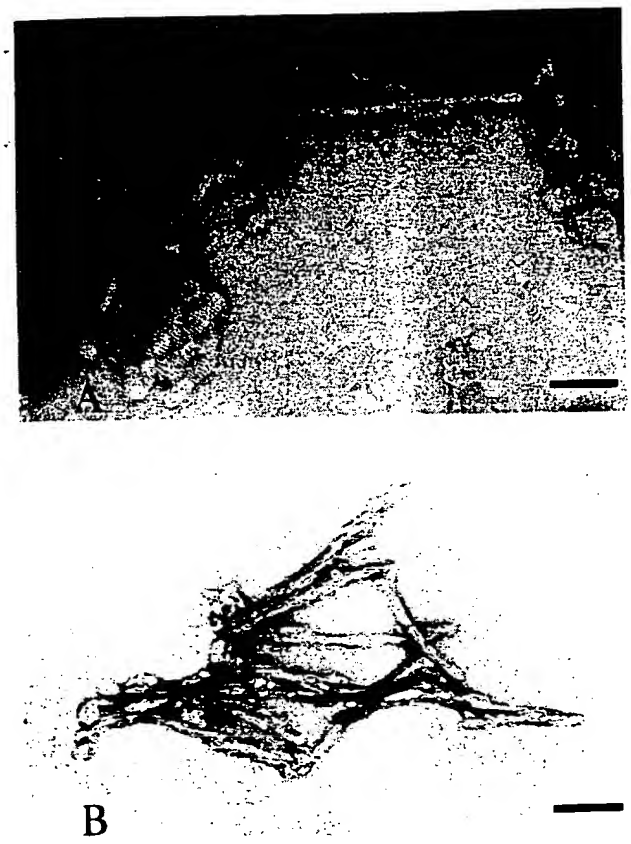

Figure 5. Electron Microscopical Visualization of Aggregates of Scrapie-Associated Fibrils Negatively Stained with 2 per Cent Uranyl Acetate.

Panel A shows fibril aggregates from the patient's brain, and Panel B fibril aggregates from scrapie-infected hamster brains (bars = 100 nm).

Discussion

The clinical history, the pathological findings in the brain at autopsy, and the demonstration of scrapie-associated fibrils and PrP_{27-30} protein established the diagnosis of Creutzfeldt–Jakob disease in this 23-year-old patient, who had for eight years been the recipient of multiple doses of growth hormone derived from human pituitary glands.

The patient was first given growth hormone in June 1969 and was maintained on standard doses until October 1977. During that time he received more than 150 vials of at least 15 different lots (A-11, A-12, A-17, A-18, A-19, A-21, A-22, A-23, A-24, B/A 18/29, 170, B-10, B-12, B-13, and B-16) of human growth hormone manufactured and distributed through the National Hormone and Pituitary Program, formerly the National Pituitary Agency. At least 9 of the 15 lots (A-11, A-12, A-17, A-19, A-21, A-23, A-24, B/A 18/29, and B-12) received by this patient were the same lots used to treat the other patient described in this issue.[1] Each lot of growth hormone that our patient received had been prepared before 1977. Eleven of the 15 lots (A-11, A-12, A-17, A-18, A-19, A-21, A-22, A-23, A-24, B/A 18/29, and 170) were prepared by the method of Wilhelmi,[9] and the remaining 4 (B-10, B-12, B-13, and B-16) were prepared by the method of Raben.[10] Neither method used procedures that would have excluded the agent of Creutzfeldt–Jakob disease from the final product or sterilized (inactivated) the Creutzfeldt–Jakob disease virus. The Wilhelmi method for preparing human growth hormone from both cold acetone-preserved and freshly collected glands consisted essentially of a series of three acetone and ammonium sulfate extractions interspersed with low-speed centrifugations. The first extractions were designed to bring into solution follicle-stimulating hormone, luteinizing hormone, and thyrotropin. The residues were then re-extracted under slightly different conditions to obtain human growth hormone and part of the prolactin. Finally, a third extraction, under different conditions of pH and temperature, was carried out to obtain the remainder of the prolactin, additional human growth hormone, and ACTH.

In his preparation, Raben[10] used acetone-extracted, dried pituitary powder, which was further extracted with glacial acetic acid at 70°C, removal of an acetone precipitate followed by precipitation of a crude fraction with ether, removal of corticotropin and intermedin from a weak acetic acid solution with carboxyl group oxycellulose, removal of a pH 8.5 precipitate, and final precipitation of human growth hormone with ethyl alcohol. Although Raben correctly stated that "treatment of the pituitary powder with acetone, ether and hot glacial acetic acid provided strong bactericidal and viricidal action in the extraction of human pituitaries of indeterminate origin,"[10] the highly resistant agent of Creutzfeldt–Jakob disease might easily have escaped inactivation.

Analytical gel filtrations on Sephadex G-100 columns of representative lots of the Wilhelmi and Raben preparations were shown to contain large amounts of aggregates and dimers, and only about 22 per cent of the preparation was monomer of human growth hormone (Parlow A: personal communication). Although lots of human growth hormone prepared by Parlow, using a revised method of extraction introduced in 1977, contain more than 95 per cent monomer and only low levels of aggregates and dimers, the procedure does not include steps known to exclude and inactivate agents causing the subacute spongiform virus encephalopathies, or as they are more commonly referred to, the "unconventional viruses."

Progress in the purification of scrapie led to the identification of scrapie-associated fibrils[11] and subsequently to the isolation and identification of an associated protein with a molecular weight of 27 to 30 kilodaltons, designated PrP_{27-30}.[12] Rabbit antibodies against PrP_{27-30} were found to react with PrP_{27-30} on immunoblots and by immunoelectron microscopy with preparations of scrapie-associated fibrils.[13] These procedures have provided laboratory methods for the detection of additional markers for the diagnosis of Creutzfeldt–Jakob disease and related diseases. Scrapie-associated fibrils have to date not been detected in tissues other than those obtained from human

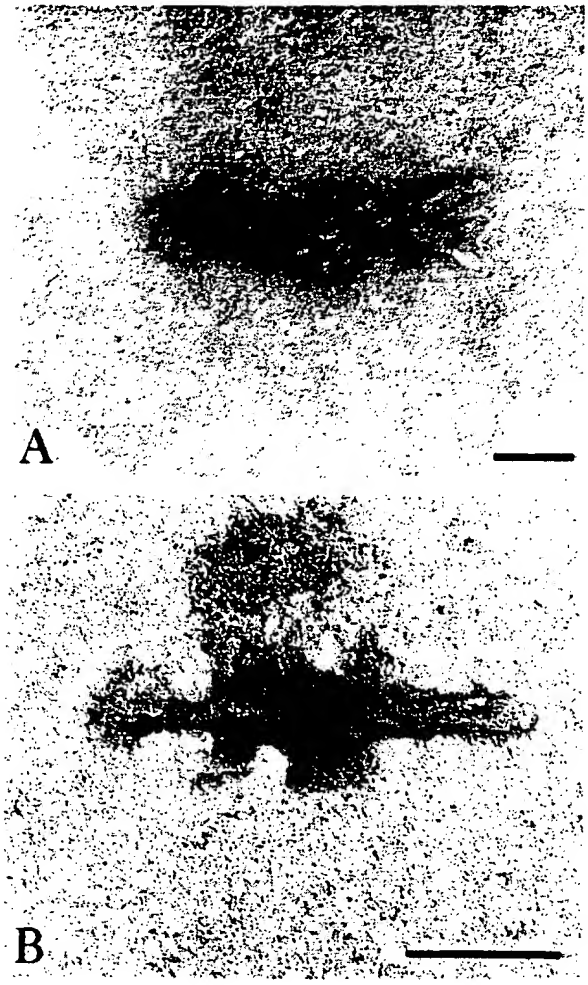

Figure 6. Immunoelectron Microscopy of Aggregates of Scrapie-Associated Fibrils.

Scrapie-associated fibril preparations from the patient's brain and from scrapie-infected hamster brains on carbon-coated grids were reacted first with a 1:100 dilution of rabbit antiserum to hamster scrapie-associated fibrils and then with sheep antirabbit IgG–colloidal gold complex. Scrapie-associated fibrils from both the patient's brain (Panel A) and scrapie-infected hamster brain (Panel B) were decorated with anti-scrapie-associated fibril rabbit-antibody–coated gold particles (bars = 100 nm).

beings dying with kuru, Creutzfeldt–Jakob disease or the Gerstmann–Straussler variant of Creutzfeldt–Jakob disease and animals dying with scrapie.[11] The gene for the PrP protein has been shown to be present in normal brain tissues; however, PrP_{27-30} has been identified only in the subacute spongiform virus encephalopathies, although an antigenically related larger protein, not resistant to proteinase K, is present in normal brain tissues. No inference should be made that scrapie-associated fibrils or PrP_{27-30} proteins are themselves the infectious agents, but their diagnostic importance remains the same. This was apparent in the case of our patient, whose diagnosis, initially questioned by a number of pediatric endocrinologists, was firmly established by the demonstration of pathological lesions consistent with Creutzfeldt–Jakob disease and of scrapie-associated fibrils and PrP_{27-30} in his brain, which gave positive reactions when tested with rabbit antiserum directed against scrapie-associated fibrils, by Western immunoblots and immunoelectron microscopy.

The pituitary gland from which human growth hormone was prepared is the probable source of the agent that infected our patient with Creutzfeldt–Jakob disease. Although attempts have not yet been made to demonstrate the infectious agent of Creutzfeldt–Jakob disease in pituitary glands of patients, the neurohypophysis and adjacent hypothalamic structures are known to be involved in the degenerative process both in patients and in animals with the disease.[14] In scrapie, the similar spongiform encephalopathy of animals, the hypothalamus and pituitary are also strikingly involved,[15] and the gland is known to be infectious.[16,17]

All 15 lots of human growth hormone received by the patient during the period 1969 through 1977 were made available to us, and these have been inoculated intracerebrally and peripherally (intravenously and intramuscularly) into juvenile male chimpanzees and individually by the same routes into three squirrel monkeys per lot. The minimum incubation period for Creutzfeldt–Jakob disease is about 1 year in chimpanzees and from 1½ to 2 years in squirrel monkeys. However, low doses may be associated with an incubation period exceeding 9 years in chimpanzees and 3 years in squirrel monkeys.

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NIDDK Fact Sheet

Human Growth Hormone and Creutzfeldt-Jakob Disease

In 1958 scientists demonstrated that children who were deficient in growth hormone began growing when treated with human growth hormone (hGH) extracted from pituitary glands. At the time, human pituitaries were the only source of the hormone. Pituitary hGH was used effectively for more than 20 years to help growth hormone deficient children.

From 1963 to 1985 a government funded program distributed pituitary hGH to children with short stature from hGH deficiency. This program was called the National Pituitary Agency until 1983 when the name was changed to the National Hormone and Pituitary Program (NHPP). The NHPP was set up to coordinate the collection of pituitary glands removed at autopsy and to distribute hGH and other hormones extracted from these glands. It is funded by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), a part of the Federal Government's National Institutes of Health (NIH). About 7,000 patients have been treated with the NHPP-distributed growth hormone.

In late February and early April of 1985, government health officials were notified that first one, and then two more men in their twenties and mid-thirties who had been treated years before with human growth hormone supplied by the NHPP, had died. All three men had symptoms of a neurologic disorder called Creutzfeldt-Jakob disease (CJD). Pathologists confirmed this diagnosis in all three cases by careful microscopic examination of brain tissue from the men. Because CJD is so rare in young adults, the reports of three deaths from CJD in this patient group led scientists to conclude that the men who died had contracted CJD due to inadvertent contamination of growth hormone. The government's distribution of hGH was stopped.

Two commercial companies, Serono and KabiVitrum, also distributed pituitary-derived hGH in this country during the past decade. The methods used by the private companies to make hGH were similar to those that the NHPP used to produce hormone after 1977. At least 2,500 to 3,000 individuals are estimated to have received hormone from commercial sources. (Many of these people also received NHPP hormone.) Shortly after the NHPP stopped distribution of hGH, these companies also ceased distributing the hormone in the U.S.

Since the first three cases of CJD were identified in U.S. recipients of growth hormone, two additional cases have been reported in this country and two overseas. Both Americans had received NHPP hormone. One died of CJD, while the other died of unrelated causes but was later found to have CJD infection based on microscopic examination of the brain. One overseas case involved a person who had received pituitary hGH prepared in a laboratory supported by the British government. The other overseas case involved a person in New Zealand who had received hGH processed in a U.S.

NATIONAL INSTITUTE OF DIABETES & DIGESTIVE & KIDNEY DISEASES

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laboratory that produced hormone for the NHPP. A pooled supply of pituitary material was used to prepare hormone for distribution in the United States and New Zealand.

What is Creutzfeldt-Jakob disease?

Creutzfeldt-Jakob disease, or CJD, is a nervous system or brain disease. It is transmitted by a particle similar to a virus. This particle, or infectious "agent," is different from the viruses most of us are familiar with. In fact, it has not yet been completely characterized. Unlike most viruses, a person infected with the CJD agent may harbor the agent for many years before becoming ill. For that reason, CJD is called a "slow-virus" disease.

What are the effects of CJD?

CJD may progress differently in different people, and it may mimic other neurologic diseases. Symptoms that may be seen in CJD include difficulty in balance while walking, loss of muscular coordination, slurred speech, failing vision, and muscle jerking, rigidity or stiffness.

These physical changes--as opposed to mental ones--have been prominent in the cases of CJD reported in hGH recipients. Changes in behavior and mental capacity also occur. These changes may include dementia, inappropriate or abnormal behavior, progressive memory loss, and confusion. (Headaches are not usually a symptom of the disease.)

The symptoms of CJD are unmistakably severe and progressive. Therefore, mild, transient clumsiness, irritability or forgetfulness should not be a cause for worry. In most people with CJD, these changes progress rather rapidly over a period of several months, and the disease is usually fatal in less than a year.

Is there any treatment for CJD?

There is no treatment that will cure or slow the progress of CJD.

How is CJD transmitted?

A few people have been infected by direct contact with contaminated tissue or instruments in the course of surgical treatments. For example, CJD has been reported in an individual who received a transplant of the cornea (the clear front covering of the eye) from a person who was later found to have the disease; in a person who received a transplant of dura mater, a tissue that covers the brain and spinal cord; in people who had electrodes implanted in brain tissue, after the electrodes had been similarly implanted in the brain of a patient with unrecognized CJD; and in a few people who underwent neurosurgical procedures in which infection probably occurred from contaminated instruments.

In the vast majority of cases, the mode of transmission of CJD is unknown. It is known, however, that it is not transmitted through casual contact or through sexual intercourse, because husbands and wives of patients with CJD are not at increased risk of developing the disease.

Why do we believe that CJD is associated with growth hormone?

CJD is extremely rare. Worldwide, there is about one case per year per million people, and nearly all of these cases are in older individuals. Prior to the reports of CJD connected with growth hormone, there had been only nine cases of the disease known to have occurred in patients younger than 30 years old.

Over the years, approximately 7,000 Americans have received hGH through the NHPP for growth hormone deficiency. Because CJD is so rare in younger people, the chances of CJD occurring by coincidence in 5 out of 7,000 individuals who received growth hormone are vanishingly small (less than 1 in 1 trillion).

There is very little doubt that the five young adults who died were exposed to the CJD agent through injections of pituitary hGH. One important goal of current scientific studies is to determine whether cases of CJD in hGH recipients will be limited to those already reported.

How would the CJD particle have gotten into supplies of hGH?

For over 20 years, the only source of hGH was human pituitary glands. The pituitary is attached to the brain, the primary target of CJD. The glands were collected from cadavers, and the growth hormone was extracted using chemical procedures. Individual pituitary glands yield only small amounts of growth hormone, so that hundreds or even thousands of glands are used to make one batch of distributed hormone. While CJD is extremely rare, we believe that one or more individuals from whom pituitaries were taken to make hGH had undetected CJD. Although efforts were made to exclude pituitaries from individuals with certain infectious brain diseases such as encephalitis and meningitis, CJD was not a specific criterion for exclusion. Also, someone infected with CJD could have died of unrelated causes with no symptoms of CJD.

Does it make any difference whether someone received pituitary hGH prepared before or after 1977?

Around 1977, scientists began using methods of extracting pituitary hGH that yielded a more highly purified hormone than was produced before 1977. All five people in the United States in whom CJD was identified received hGH that was produced before 1977. One of these patients received hormone produced after 1977 as well.

While the methods of preparation of pituitary hGH used since 1977 yield a product that is more than 95 percent pure, there is no certainty that the modern preparation is safer than the hormone extracted before 1977. It is important for medications to be as highly purified as possible. The more effective the purification process, the less likely a medication is to cause allergic or toxic reactions. The infectious particle that causes CJD, however, is very small and extremely difficult to destroy or inactivate. The particles resist treatment with chemicals like formalin, hydrochloric acid, and alcohol. Extraction methods that result in 95 percent chemical purity still may not remove or inactivate the CJD agent.

In an effort to determine whether hormone extraction methods would eliminate the CJD agent, the British Medical Council performed an experiment in 1980 in which they deliberately introduced a slow virus similar to the CJD agent into growth hormone preparations. (They used the infectious particle for scrapie, a disease of sheep.) Then they purified the preparation using routine procedures for hGH preparation similar to those used by the NHPP since 1977, and the product was tested in scrapie-susceptible animals. No infection resulted, indicating that the purification steps had indeed removed most or all of the scrapie particles. Although encouraging, this study is not a guarantee that all the infectious particles had been removed or that all pituitary hGH produced after 1977 is safe.

Is there a diagnostic test for CJD?

There is no test that can determine whether a healthy person is incubating the disease. One of the goals of research on CJD is to develop a diagnostic test that can detect infection in someone with no symptoms. A spinal fluid test has recently been developed that may aid in the diagnosis of patients with symptoms of CJD. At this time, however, this test can only help confirm the diagnosis of CJD in someone who already has symptoms that suggest the disease.

Why don't we know more about CJD?

Only in the late 1950's did scientists begin to suspect that certain neurologic disorders might be transmitted by an infectious agent distinct from any known virus. It was not until the late 1960's that CJD was recognized as a condition that was transmitted by a virus-like agent. Several teams of scientists are working to identify the infectious particle that causes CJD, as well as other slow-virus diseases. There is evidence that such an infectious particle may be smaller than and unlike any virus known up to this time. Scientists are studying transmission of the disease, developing accurate methods of detecting it, and searching for methods to inactivate the slow-virus agent.

I (or my child) have been treated with pituitary hGH. What should I do now?

Stay in touch, if possible, with the physician who prescribed the growth hormone. If that is not possible, contact one of the organizations that has an interest in hGH and hGH research. The addresses of the Human Growth Foundation and the Parent Council for Growth Normality are listed at the end of this fact sheet. These groups will relay new information to members. You can also call the National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, with questions. The number is (301) 496-3583.

You can help obtain answers to questions of patients and their families about the long-term results of treatment with pituitary hGH by participating in an epidemiology study described starting on p.4 of this fact sheet. This study is likely to be the most valuable means we have of gathering information on hGH treatment and CJD.

Are there any measures a recipient of pituitary hGH may take to protect his or her health or that of others?

There is no reason for a person who has received pituitary hGH to make any changes in day-to-day living, health habits, interaction with family members and sexual activity, or general attention to his or her own health because of fear of CJD.

The only exception to this advice relates to donation of blood or of other tissues and organs for transplantation. Because there is no way of testing to detect infection with CJD, blood banks will not collect blood from anyone who has been treated with pituitary hGH.

This recommendation reflects the great caution with which the blood banking community handles selection of blood donors. It is also recommended that pituitary hGH recipients not donate other tissues or organs for transplantation.

This recommendation has no bearing on an hGH recipient's day-to-day life and interaction with others. Experts in CJD believe there is no danger of infection to family members if a person were to be incubating CJD or had active disease. Husbands and wives of patients have no increased risk of contracting the disease. CJD is not transmitted through sexual contact, and it is not transmitted from a mother to her unborn baby across the placenta.

In summary, there is no reason for someone to take any special precautions with a friend or family member who has received pituitary hGH.

What are the chances that someone who has received pituitary hGH over the years has been exposed to CJD and may become ill?

Unfortunately, there is no way for us to know that at this time. In order to answer this question, we need to know how likely it is that the CJD agent was present in the batches or lots of hGH. We need to know how likely it is that someone exposed to the agent will contract the disease and whether the dilution of the particle in growth hormone preparations affects the likelihood of infection. We also need to know if people differ in their susceptibility to contracting this disease.

To date, there have been five cases reported out of an approximate total of up to 10,000 people in this country who are believed to have received pituitary growth hormone from any source. One of the five died of causes unrelated to CJD but was found to have evidence of infection based on microscopic examination of the brain. Because CJD has a long incubation period, it is too early to tell whether additional cases of the disease will develop in persons who received pituitary hGH.

What is being done to answer these questions?

To help determine the extent of contamination of pituitary hGH by CJD, samples of all available lots of hGH used by the NHPP have been inoculated into experimental animals. The animals have been followed 2 1/2 years without signs of infection and will be watched for at least 5 years for signs of the disease. This kind of testing, called a bioassay, is the most reliable way to test for contamination with the CJD infectious agent. A negative result, however, may only mean that the particular vials tested contained no virus, whereas another vial in the same lot that was given to a patient could have contained virus.

The scientists carrying out this study are among the world's leading experts in slow-virus diseases. This group of scientists also used new techniques in an attempt to detect a protein-containing filament that is characteristic for CJD in hGH preparations and in blood taken from individuals who have had hGH. While no evidence of contamination has been found, these methods of detection are not as sensitive as the bioassay, and a failure to find contamination using them would not prove that there was no contamination.

Separate from this laboratory effort is an epidemiology study that is gathering information on the health status of as many people as possible who received hGH distributed through the NHPP. The study is aimed at determining if there have been any cases of CJD other than those that have already been identified. In addition, the study will also attempt to follow hGH recipients for several years in order to track any future cases of CJD in this group.

This study is possible because the physicians who distributed NHPP growth hormone had to keep records of patients who received the hormone. The commercial companies who marketed growth hormone once it was approved by the Food and Drug Administration (FDA) were under no obligation to keep records of the users of growth hormone--just as they are not required to keep records of users of other prescription drugs. However, many recipients of pituitary hGH from commercial sources may be traced through the epidemiology study because many of these individuals received NHPP hormone as well as the commercial product.

Through the epidemiology study, we hope to provide patients who received pituitary growth hormone with an estimate of their risk of developing CJD. Two of the NIH Institutes, the NIDDK and the National Institute of Child Health and Human Development, are funding this study.

Scientists from the government's Centers for Disease Control (CDC), the FDA, and NIH, together with a panel of advisors from the academic community who are experts in pediatric endocrinology, neurology, virology, and epidemiology, are participating in designing and

carrying out the study.

I understand that a biosynthetic form of hGH is available. How is it different from the previously used hGH?

Cells in the human body make hGH using inherited instructions. These instructions, or genes, are composed of the molecule DNA. Scientists have succeeded in inserting the DNA sequence that determines the structure of hGH into the DNA of bacteria. The bacteria are a type found normally in the human digestive tract. By a process called biosynthesis, the bacteria make hGH using the inserted human DNA as a blueprint. The hormone is purified so that no bacteria are present in the hormone used for treatment.

In 1985 the FDA approved the first biosynthetic growth hormone for use in children with growth deficiency. This hormone is identical to human growth hormone except for one extra amino acid. (Amino acids are links in the chemical chains that form proteins--hGH is a protein that is 191 amino acids long.) In March 1987, a second form of biosynthetic growth hormone that does not have the extra amino acid, became available commercially. Other companies are continuing to develop new biosynthetic forms of hGH.

Is biosynthetic growth hormone as effective as pituitary hGH?

In order for a drug, in this case biosynthetic hGH, to be approved by the FDA, the firms that market the drug perform clinical tests to establish that it is both effective and safe. In tests in which patients with growth hormone deficiency were treated with one or the other type of biosynthetic hGH, biosynthetic growth hormone stimulated growth in exactly the same manner and to the same extent as pituitary hGH.

Is biosynthetic hormone safe?

Because biosynthetic hGH is not made from human tissue, there is no chance of contamination with the CJD infectious particle.

Clinical tests of biosynthetic hGH before it was marketed sought to identify any adverse effects associated with the hormone. In particular, it was important to know whether recipients would react to the extra amino acid in the first available form of biosynthetic hormone, or to the minute amounts of impurities left after the biosynthetic process in either type of biosynthetic hGH.

A person's immune or disease-fighting system can recognize substances that are not identical to anything the body itself produces. The immune system may respond to a foreign substance, such as a hormone, by making antibodies to remove or inactivate it. There was some concern that antibody response might inactivate the biosynthetic growth hormone.

Although antibody formation sometimes occurs, only very rarely do the antibodies interfere with the growth-promoting effect of biosynthetic growth hormone. Antibody formation stops when biosynthetic hormone is stopped. (Antibody formation also occurred with pituitary hGH, usually without effect, but occasionally interfering with the hormone's ability to promote growth.)

As research continues, production methods improve. The biosynthetic hormones now being used are more highly purified than the first such hormone that was clinically tested. In other words, they contain more hormone relative to the very small amount of material that remains from the biosynthetic process. Children in the clinical tests of these more highly purified hormones have had lower levels of antibodies to injected hGH than were found in the first clinical trials with biosynthetic hGH.

In summary, no major or health-threatening side effects have been associated with biosynthetic hGH use.

We were told that pituitary hGH was safe, too. How can we be sure that biosynthetic hGH is safe?

When the FDA approves drugs for use, the decision is based on the best available knowledge of the risks and benefits of the medication. This judgment is made within the limits of the capabilities of clinical testing and current understanding of how medications work. Unfortunately, it is not possible to guarantee that any medication will be 100 percent safe.

However, at least 6 years of records beginning from the first patient tests of biosynthetic hGH are now available. From this evidence, there is no reason to believe that biosynthetic hGH will cause serious or long-term health problems.

Will the NHPP distribute biosynthetic hGH at no charge for patients in research studies as the program did with pituitary hGH?

No. When the NHPP was established, the only source of hGH was human pituitaries. Because of the limited supply of human pituitaries, a central resource was needed to ensure that the largest possible number of glands were collected and that hormones were systematically extracted and made available

to patients for treatment under approved research protocols. With the development of biosynthetic hGH, the supply of hGH is no longer limited.

Years of clinical research have documented the effectiveness of hGH in stimulating the growth of children with hypopituitarism. There are still important needs for research with growth hormone, and NIH continues to support research in this area. NIDDK continues to distribute pituitary-derived growth hormone to scientists for nonclinical laboratory research. NIDDK does not plan to purchase biosynthetic growth hormone for distribution for clinical research. Drug companies provide biosynthetic hGH to scientists who are working on specific clinical research projects.

Can biosynthetic hGH be covered under Medicaid or private health insurance?

Human growth hormone is an FDA-approved drug. Under Federal guidelines, approved drugs can be covered under Medicaid benefits. Each state has its own guidelines for eligibility for health benefits. The best way to investigate this coverage is to contact the state health or social services department, or Medicaid office.

Private insurance coverage depends on the individual policy. If you have not already done so, discuss insurance coverage of biosynthetic hGH treatment with your insurance carrier and the doctor responsible for your child's treatment.

Additional Reading

A list of papers published in the scientific literature on hGH and CJD is available on request from the National Institute of Diabetes and Digestive and Kidney Diseases (see address below).

Other Resources

If after reading this fact sheet, you have concerns or questions, please call:

National Institute of Diabetes and Digestive and Kidney Diseases
Building 31, Room 9A04
Bethesda, Maryland 20892
(301) 496-3583

Other resources on the subject include:

Food and Drug Administration
Division of Metabolism and Endocrine Drug Products
(HFN-810)
Center for Drugs and Biologics
5600 Fishers Lane
Rockville, Maryland 20857
(301) 443-3510

Human Growth Foundation
Montgomery Building
4720 Montgomery Lane
Bethesda, Maryland 20814
(301) 656-7540

Parent Council for Growth Normality
2899 Camelia Drive
Opelousas, Louisiana 70570
(318) 942-9700

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A PRISMATIC CASE

The Prismatic Case of Creutzfeldt-Jakob Disease Associated with Pituitary Growth Hormone Treatment

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Since 1984, a total of 15 definite cases of Creutzfeldt-Jakob disease (CJD) have been associated with the use of GH purified from pituitaries in the United States, and a total of at least 60 cases have occurred worldwide. It is possible, perhaps likely, that other cases of CJD will continue to appear in the future. This is a personal account of the clinical presentation of the index case, and how this led to the eventual withdrawal of pituitary GH from use world wide.

Case report

On Sunday, June 17, 1984, I received a phone call at home from the mother of JRo. JRo was a 20-yr-old man who had been treated for GH deficiency and insulin-dependent diabetes mellitus at Stanford University and before that at Los Angeles Children's Hospital. He had recently "graduated" to the adult endocrine clinic. However, the parents still regarded me as his doctor and called me because they were worried about a change in his behavior. JRo had been away from home for a period of time visiting relatives, and when he got off the plane the mother noted that he was clumsy and had awkward movements. Because I knew these parents were good observers and they were very concerned, I made arrangements to meet them at the emergency room. On examination, I became convinced that JRo did have definite ataxia and arranged for him to be seen by a neurologist. At the time of this emergency room visit, I did not imagine that this mild ataxia would lead to his death within 6 months and the withdrawal of human pituitary GH from therapeutic use in the United States within 12 months.

In many ways this patient was a unique case. He presented in infancy to Dr. Maurice Kogut and his colleagues at Los Angeles Children's Hospital with a decrease in growth rate and secondary hypothyroidism, and then developed severe hypoglycemia followed by the onset of insulin-dependent diabetes mellitus. Because of severe hypoglycemia, insulin sensitivity, and persistent growth failure, he was diagnosed early in life as having profound GH deficiency and was started on GH treatment before the age of 3 yr (1). After the parents moved to northern California, JRo received his pediatric endocrine care at Stanford University. It became apparent that any attempt to manage him with GH every other day or three times a week, which was then the custom in the United States, would be impractical because of the wide swings in his insulin requirements between the days when he received GH and the days when he did not. Thus, JRo became a pioneer in the receipt of daily GH in fairly large doses over a long period of time. In response to this treatment and a subsequent increase in GH dosage, his growth rate was quite good, and his final attained height was above 165 cm (Fig. 1). In many ways, JRo was regarded by his doctors and his family as a medical success. However, the onset of ataxia when he was 20 yr old became the harbinger of a series of disastrous neurological events that progressed rapidly over a few weeks span. The parents were obviously alarmed at his downward course and sought other neurological opinions. He developed severe dementia in addition to his ataxia, deteriorated to the point that he needed hospitalization, and died within 6 months of the onset of symptoms. Before his death in November 1984, a number of diagnostic possibilities were considered, including CJD, but no certain conclusion was reached. However, on autopsy, the classic pathological findings of spongiform encephalopathy diagnostic of CJD were found (2). I had kept in contact with the parents of JRo and his neurologists, Dr. Gravina, Dr. Berg, and colleagues at the University of California at San Francisco, throughout his disastrous clinical course. When I was informed that the postmortem diagnosis of CJD was certain, I was very concerned. I had attended a conference on standards for insulin, somatotropins, and thyroid axis hormones sponsored by the FDA in 1982. One of the topics that was discussed informally during the meeting was slow virus disease and the difficulty of detecting contamination in tissue extracts.

Because CJD was such an unusual disease in a young person, I was very concerned that the CJD of JRo might have come about from contamination of pituitary GH supplies by the CJD infectious agent. If this were true, all of the patients that we had treated with pituitary GH in the previous 2.5 decades might be at risk of developing this disastrous degenerative neurological disease. I, therefore, believed it was important to bring this case to the attention of the endocrine community at once. On February 25, 1985, I wrote a letter describing JRo and his unusual development of CJD and sent it simultaneously to the FDA, the National Hormone and Pituitary Program (NHPP), and the NIH. The letter stated:

"Creutzfeldt Jakob disease is extremely rare in a 20-year-

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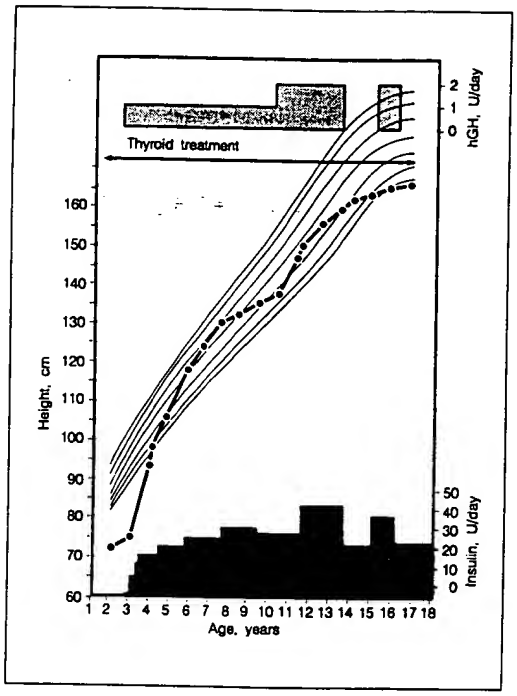

FIG. 1. Growth pattern and treatment of patient JRo. Data are derived from Ref. 1 and Stanford University Medical Center records (22).

old, and it is felt to be transmissible by contact with the infectious agent in the central nervous system of affected patients. This patient (JRo) was treated for 14 years with growth hormone, and I feel that the possibility that this was a factor in his getting Creutzfeldt Jakob disease should be considered. A careful follow-up of all patients treated with pituitary growth hormone in the past 25 years should be carried out, looking for any other cases of degenerative neurological disease. I am personally unaware of any such events happening, but the potential seriousness of this situation has led me to report it to you."

The response to my letter was prompt. Dr. Mortimer Lipsett, Director of the NIDDK, who was responsible for the oversight of the NHPP, convened a meeting of officials and scientists on March 8, 1985 to consider the problem (3). It was decided to notify physicians about the potential problem, to get more information about JRo, and to initiate a search for similar cases among GH-treated patients. Suspension of the therapeutic use of GH was not done, pending further information. I remember the next 6 weeks as a stressful time. Although my colleagues at Stanford were fully supportive of my action, many of my other pediatric endocrine colleagues clearly felt that I was an alarmist, and they did not hesitate to tell me so. The pressure increased when it appeared that the therapeutic use of GH in the United States might be halted because of this apparently unique case. Thus, more than 3000 patients with GH deficiency would no longer be able to receive needed treatment because of my single case report. A meeting to consider the fate of the therapeutic use of human pituitary GH was organized to take place at the NIH on April 19, 1985. This meeting included representatives and scientists from the NIH, FDA, NHPP, plus the pharmaceutical industry and pediatric endocrinologists from several countries. I was one of the invited attendees. When I arrived in Bethesda on the late afternoon of April 18, 1985, I met with Drs. Robert Blizzard and Selna Kaplan. It was at this point that I learned from them that two other probable cases of CJD in patients treated with pituitary GH in the United States (4, 5) had been reported to the NIH in the preceding week. By an incredible coincidence, these pituitary GH-treated cases had died of neurological disease within the previous few months. In light of my report of JRo, Dr. Blizzard and Dr. Margaret MacGillivray had reviewed these cases and realized that they were strikingly similar in their clinical presentation and inexorable fatal course to the autopsy proven case of CJD that I had reported. In the light of this crucial new information, the meeting the next day was a foregone conclusion. Despite efforts by some of the participants in this meeting to depict the problem as isolated to the NHPP and its purification methods or as a strictly American problem, the therapeutic use of all human pituitary GH, both that distributed by the NHPP and that purified abroad and marketed in the U.S. by pharmaceutical companies, was effectively ended in the United States on April 19, 1985.

Aftermath

It is quite clear now that this devastating disease is undoubtedly associated with the use of pituitary GH. In addition to the unique cluster of young patients with CJD among pituitary GH-treated patients, one of the monkeys who was injected with suspected lots of pituitary GH has developed CJD after a long incubation period (6). Recent information suggests that allelic homozygosity at codon 129 of the chromosome 20 amyloid gene is an additional risk factor in susceptibility to the CJD infectious agent (7). Thus, the GH-deficient patients who developed CJD may have had not only an unusual iatrogenic exposure to CJD infectious agent, but also a genetic susceptibility to the development of CJD. There have now been a total of 15 cases of CJD identified in patients treated with pituitary GH prepared in the United States (Brown, P., personal communication). Of these, 12 have occurred in the United States (8–11), whereas 1 patient in Brazil (12) and 2 patients in New Zealand (13) were associated with the use of pituitary GH prepared in the United States. A thorough survey of the vast majority of pituitary GH-treated patients in the United States (14) failed to reveal any cases of CJD not already identified, but further periodic follow-up of these patients is planned.

So far, all of the cases identified in the United States had treatment with pituitary GH initiated before 1970, although it is unclear whether this is due to the long incubation time of this disease or a change in the early 1970s in the purification procedures for pituitary GH used by the NHPP. The time between the initiation of treatment with pituitary GH and the development of the symptoms of CJD in the first nine patients who developed symptoms before death ranged from 16–34 yr, with a mean of 22.6 yr, and the duration of GH treatment was as short as 2 yr (Table 1). Thus, many patients who received pituitary GH treatment in the United States are still within the wide range of the observed incubation period of CJD. A number of the cases of CJD occurring outside the

TABLE 1. Characteristics of the first 10 patients to develop CJD associated with U.S. pituitary GH

Date GH started	Duration of Rx (yr)	Time to symptoms (from start of Rx, yr)	Age at death (yr)
1957	9	34	41
1959	13	28	32
1962	6	23	32
1963	2	22	37
1965	14	19	20
1965	17	22	26
1967	9	20	28
1968	11	[a]	16
1968	9	16	22
1969	3	19	32

[a] This patient died before the onset of symptoms.

Data derived from Refs. 5 and 10, and minutes of the pharmacy and therapeutics committee of the Lawson Wilkins Pediatric Endocrine Society.

United States had their GH treatment initiated well after 1970. The GH purification method used in these countries was similar in stringency to the NHPP methods used after the early 1970s. Certainly, it is still too soon to define the scope of this complication of treatment with pituitary GH, and more cases are likely to appear in the future. After a nearly 2-yr hiatus of new cases in the United States, one new confirmed case and one probable case of CJD have been reported in the last 6 months. Thus, the development of new cases of CJD in patients treated with pituitary GH in the United States seems likely to smolder on for some time to come.

Worldwide experience

Soon after the initial case reports of the association between CJD and pituitary GH, and the withdrawal of pituitary GH from therapeutic use in the United States, a single case was reported in Great Britain (14). It thus became apparent that this problem was not limited to the GH prepared from pituitary glands in the United States. A total of 15 cases of CJD associated with the use of pituitary GH have now been reported from Great Britain (Preece, M., personal communication) (15, 16), and an additional case is under investigation. Another case of CJD associated with the use of pituitary GH was reported from France in 1991 (17). A survey of all cases treated with pituitary GH in France revealed several additional cases (18). The French cases are very different from the experience in the United States and the United Kingdom, with a much shorter incubation time and a more recent onset of pituitary GH therapy. The data suggest that the contamination in the French purification of pituitary GH may have occurred between 1983 and 1985. There are now reported to be 34 definite cases of CJD in France associated with pituitary GH treatment (Agid, Y., personal communication), and legal action has been initiated (19). At the time of this writing, a total of at least 60 cases of CJD in patients treated with pituitary GH have been identified worldwide.

Perspective

Treatment with human pituitary GH was first reported in 1958 (20). After the organization of the National Pituitary Agency in 1962, the surge in the use of GH in the United States was almost logarithmic for the next 2 decades (Fig. 2). By 1985, approximately 3000 patients in the United States were receiving treatment with human pituitary GH. Estimates in the early eighties suggested that there were approximately twice as many GH-deficient patients who deserved treatment than were being treated in the United States at that time (21).

Several months after the use of pituitary GH in the United States was stopped in early 1985, the use of synthetic GH was approved by the FDA in the United States. By the end of 1986, the number of patients receiving GH treatment in the United States had doubled to more than 6,000, and a steady increase in the use of synthetic GH has continued. At present, more than 20,000 patients are currently being treated with GH. It is estimated that since 1960 nearly 40,000 children in the United States have been treated with GH of either pituitary or synthetic origin. During this period of time, a number of unfavorable clinical events associated with GH therapy have been reported in addition to the association of pituitary GH with CJD (22). Some of these events have been clearly treatment related, but others are of unclear etiological association with GH treatment. Although 40,000 children is a relatively large number, this treatment experience is not large enough to determine whether other rare unfavorable events might be etiologically linked to synthetic GH treatment.

Conclusions

The risk of CJD makes any further therapeutic use of human pituitary GH purified from pituitary glands unjustified (17), and essentially all therapeutic use of human pituitary GH has halted worldwide. Overall, the use of synthetic GH has been associated with remarkably few

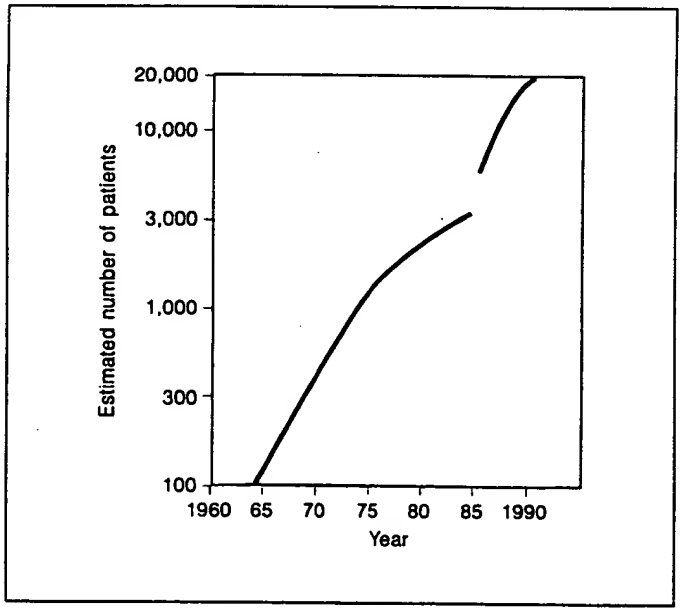

FIG. 2. Estimated use of GH in the United States. The number of patients estimated to currently be receiving treatment with GH in the United States from 1964 until the present. Data are derived from National Hormone and Pituitary Program data (2) and commercial sources. The estimated number of patients receiving active treatment is plotted on a logarithmic scale (22).

problems. However, it needs to be kept in mind that GH is a very potent biological agent. As the therapeutic indications of GH treatment widen in both children and adults, and the dosages of GH increase, more unfavorable clinical events linked directly to the biological actions of GH can be expected to occur. In addition, the occurrence of rare (1 of 10^4 to 10^5), but clinically significant, associations between GH and unfavorable clinical events may have been missed in the approximately 60,000 patients in the world that have been treated with GH to date. Thus, constant surveillance for rare, but important, associated events must continue, and each endocrinologist must be alert to the occurrence of unfavorable clinical events during the use of GH therapy. The organization and maintenance of surveillance studies for potential complications must be an important concomitant to the widening use of GH therapy in the United States and around the world.

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
National Institutes of Health

National Institute of Diabetes and
Digestive and Kidney Diseases
Bethesda, Maryland 20892

OCT 28 1987

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Dear Colleague:

I want to make you aware of recent discussions among leaders in the blood banking community on the issue of whether patients who received pituitary-derived growth hormone should serve as blood or tissue donors. It appears there may be a remote risk that if a growth hormone recipient harbored the Creutzfeldt-Jakob Disease (CJD) agent, it could be transmitted through blood transfusion. Infectivity has been demonstrated in blood from a few patients who were already ill with CJD, although whole units of blood from a few other patients with CJD were not infectious. Moreover, animal studies have shown that CJD can be transmitted from blood of infected animals before the development of signs of illness. A panel of experts on CJD felt that the possibility for transmission of CJD through donated blood from growth hormone recipients was very remote but did exist. Given the unwillingness to accept a preventable risk of transmission of disease through the blood supply, the blood banking organizations jointly decided to exclude pituitary growth hormone recipients from blood donation.

I recognize the concern this decision may cause in the growth hormone recipients and their families and would like to communicate it with sensitivity. Ideally I would prefer that information come from the treating physician.

Because media coverage of issues related to the blood supply is intense, it is possible that the decision that growth hormone recipients should not donate blood will receive inappropriate media attention. As you remember, many patients first learned about the possible contamination of growth hormone with the CJD agent from the media. When informed about the recommendation on blood donation, parents' groups have urged the Public Health Service to notify patients directly so that the recommendation can be accurately presented in an appropriate context. We will include this recommendation in a revised Fact Sheet for distribution to patients.

I would like to take this opportunity to keep you informed on other developments in this area. Since the first three deaths from CJD in the U.S. were identified in 1985, one other patient became ill and died of CJD in the U.S. as did one patient in New Zealand. The New Zealand patient received growth hormone prepared in the same U.S. laboratory which produced NHPP hormone and there was admixture of U.S. and New Zealand pituitaries before preparation of separate batches of hormone for distribution in the two countries. Growth hormone received by an English patient who developed CJD was prepared in Great Britain and was completely

unrelated to hormone distributed in the U.S. In a seventh patient, who had died years earlier of viral pneumonia, pathologic changes diagnostic of CJD were found upon reexamination of brain tissue. This U. S. patient had no clinical signs or symptoms of CJD, but the pathologic findings indicate she harbored the infectious agent at the time of her death.

In 1985 the Public Health Service initiated two sets of studies to determine the extent of the problem. First samples of all available batches of growth hormone distributed through the NHPP were inoculated intracerebrally into primates. The incubation time after intracerebral inoculation with CJD is approximately two years, but may be longer with low titer inoculums. These animals have now been followed for 2 1/2 years without evidence of infection. The animals will be followed for at least five years. A negative result does not exclude contamination since not every vial would necessarily include infectious agent.

Second, the Public Health Service is conducting an epidemiologic followup of all NHPP growth hormone recipients. In 1985 we believed that approximately 10,000 children had been treated, however we did not have an actual list of names. As identification of these patients has progressed, we have found many duplicate listings and now believe the number treated was about 7,000. Thanks to the cooperation of the pediatric endocrinologists treating these children, we have been able to identify just over 6,000 patients who received growth hormone from the NHPP. The identification of these patients, sometimes from records over 20 years old, has been a very difficult and time consuming activity. I want to personally thank you for the effort you have devoted to this undertaking.

We are currently following up on all known deaths in this patient population; both deaths reported by endocrinologists and deaths discovered by matching the patients' names against the National Death Index. Medical records on each patient who died will be reviewed to determine whether the patient had signs or symptoms suggestive of CJD. All available brain pathology is being reviewed by neuropathologists with expertise in CJD. So far no cases of CJD other than those described above have been found. We also plan to conduct a telephone interview to determine the health status of each growth hormone recipient. We anticipate that the interviews will begin in early 1988 and will take about eight months to complete.

Overall, the events of the past two and one half years have not confirmed early fears of the potential magnitude of the CJD problem and I think the basic message to patients should be one of hope and encouragement.

Sincerely,

Phillip Gorden, M.D.
Director

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
National Institutes of Health

National Institute of Diabetes and
Digestive and Kidney Diseases
Bethesda, Maryland 20892

January 10, 1994

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Dear Colleague:

I am writing to provide you with current information on the status of the problem of Creutzfeldt-Jakob disease in pituitary growth hormone recipients. As in the past, we are providing this information to physicians who treated patients with hormone from the National Hormone and Pituitary Program (NHPP) prior to sending it directly to hormone recipients. It is important that you have the opportunity to review the enclosed materials should your patients call you with questions or concerns after receiving it. We will be sending this information to adult hormone recipients and parents of hormone recipients within the next few weeks.

If you would like extra copies of the material or have any questions, you may contact the Institute's Information Office:

National Institutes of Health
NIDDK Information Office
Building 31, Room 9A04
Bethesda, MD 20892
(301) 496-3583

If you wish to speak with a physician coordinating the follow-up study, you may also contact:

Judith E. Fradkin, M.D.
Chief, Endocrinology and Metabolic
Diseases Programs Branch
Division of Diabetes, Endocrinology and
Metabolic Diseases
Westwood Bldg. Room 621
NIDDK, NIH
Bethesda, MD 20892
(301) 594-7567

NIDDK will continue to provide periodic updates on the status of NHPP growth hormone recipients and we hope you find this information helpful.

Sincerely,

Phillip Gorden

Phillip Gorden, M.D.
Director

INFORMATION FOR RECIPIENTS OF NHPP GROWTH HORMONE

What is the total occurrence of CJD among NHPP growth hormone recipients?

When the follow-up study began, the Public Health Service was able to identify 6,284 patients known to have received NHPP growth hormone. There were approximately 2,000 other patients for whom physicians had requested hormone from the NHPP, but the physicians could not confirm growth hormone treatment or provide information needed to identify and contact the patients. Although these patients could not be included in the interview study, the Public Health Service believed that we might learn of any cases of CJD that developed in these patients due to the wide dissemination of information about the problem of CJD to physicians. This has proven to be the case since three of the eleven cases of CJD identified among NHPP growth hormone recipients were reported among the 2,000 patients who could not be confirmed as treated when the study began.

There have now been eleven cases of CJD among approximately 8,000 NHPP growth hormone recipients, or one case for every 700 people who received NHPP growth hormone. Since onset of the problem of CJD in growth hormone recipients, the rate of occurrence of new cases has been about one per year.

What are the symptoms of CJD?

In growth hormone recipients who have developed CJD the first symptoms have generally been unsteadiness while walking, difficulty with balance, dizziness, and clumsiness. Other symptoms that usually occur later include slurred speech, problems with vision, muscle jerking, rigidity or stiffness, or problems with memory or thinking clearly. The symptoms of CJD progress rapidly and within two to three months usually become unmistakably severe. Therefore, mild transient clumsiness, irritability or forgetfulness should not be a cause for worry. Headaches are not a symptom of the disease.

How long after growth hormone treatment can CJD occur?

Most of the patients who developed CJD received growth hormone for many years. The interval from the midpoint of hormone treatment to development of symptoms of CJD has averaged 17 years in the United States. Among Americans, CJD has occurred from as few as 4 years after stopping hormone therapy to over 30 years after starting therapy.

Are patients who received pituitary growth hormone produced by newer methods introduced in 1977 at risk for CJD?

There have still been no cases of CJD among Americans who began growth hormone therapy after newer methods of hormone purification were introduced in the United States in 1977. The method used after 1977 included a chromatography purification step that markedly reduces any CJD infectious material contaminating the pituitaries from which growth hormone was extracted. Each year that goes by without occurrence of CJD in patients who started treatment after the newer hormone was introduced provides encouragement about the safety of these preparations. However, more time must past before we can draw definite conclusions about the safety of hormone produced for the NHPP after 1977, due to the long incubation period for CJD.

Has a particular growth hormone preparation been identified as the source of CJD?

In 1985 the Public Health Service tested all available preparations of NHPP growth hormone for ability to transmit CJD to animals susceptible to CJD. Transmission of CJD to an animal would prove the growth hormone was infected with CJD. However, lack of transmission would not be proof that the hormone was not infectious, since not every vial of hormone might contain enough infectious material to cause disease. The time between injection of infectious material and onset of CJD in animals is usually less than three years, but when the level of infectious contamination is very, very low, the incubation time can be prolonged. Only one animal has developed CJD, five and one half years after injection with hormone. None of the patients who developed CJD is known to have received the particular growth hormone preparation that transmitted CJD to the animal, although it is possible that one patient (whose records are incomplete) may have received this hormone preparation. We do not believe that patients who received this particular preparation are at increased risk compared to other growth hormone recipients. Rather, based on our analysis of the preparations received by the patients who developed CJD, we continue to believe that multiple preparations of hormone contained very low level contamination with CJD infectious material. We believe that the contaminated preparations had so little infectious material that most vials from these preparations would not transmit CJD.

Is it possible to predict who will develop CJD?

Researchers at the National Institutes of Health are continuing to investigate the possibility that susceptibility to CJD may vary among patients who received growth hormone. There are genetic variations in the structure of the brain protein that becomes abnormal in CJD, and it is possible that these genetic variations affect the risk of developing CJD. In the United States there are not enough patients who developed CJD after receiving growth hormone to test this possibility. Material from French and British hormone recipients who developed CJD is also being analyzed, but we do not have a test that can identify hormone recipients who may be at increased risk.

Have growth hormone recipients in other countries developed CJD?

Development of CJD following pituitary hormone administration has occurred in many countries. The problem of CJD has been particularly severe in France with 25 cases of CJD among approximately 1,700 growth hormone recipients. The pattern of exposure to CJD is very different in France than in the United States. In the United States there is no clustering of cases linked to any particular hormone preparation. In France the risk of CJD appears linked to treatment during 1984-5, suggesting people who received particular hormone preparations distributed during this time period might be at increased risk. The greater number of cases and shorter time between treatment with growth hormone and onset of CJD in France compared to the United States suggests a higher level of infectious material in the contaminated French growth hormone preparations compared to the United States. In England there have been eleven cases of CJD among approximately 1,900 growth hormone recipients. There have also been single cases of CJD following growth hormone treatment in Brazil and New Zealand, and four cases in Australian women who received other pituitary-derived hormones.

Have any other problems been identified in growth hormone recipients?

Investigators in Japan found that growth hormone recipients were at increased risk for leukemia. Unlike CJD, the cases of leukemia in Japanese growth hormone recipients generally occurred within three years of hormone therapy. There have not been reports of increased risk of leukemia among growth hormone recipients from any other country. The United States Public Health Service investigated the possible relationship of growth hormone administration to risk of leukemia among NHPP growth hormone recipients. Two to three cases would be expected among these patients based on general rates of leukemia in the U.S. population. Six patients developed leukemia. Five of these six patients had brain tumors as the cause of their growth hormone deficiency and four of them had received radiation treatment. Their risk for leukemia may have been increased by conditions such as tumors or radiation treatments. There was no increase over the number of cases of leukemia expected in the majority of growth hormone recipients who did not have brain tumors or radiation treatments. Thus, the higher rate of developing leukemia in growth hormone recipients may be due to factors other than growth hormone.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
National Institutes of Health

National Institute of Diabetes and
Digestive and Kidney Diseases
Bethesda, Maryland 20892

February 13, 1991

Dear Growth Hormone Recipient:

When the U.S. Public Health Service began the epidemiologic follow-up study of recipients of pituitary-derived human growth hormone supplied through the National Hormone and Pituitary Program (NHPP), you asked to be kept informed of the progress of the study and of new developments with regard to growth hormone administration and Creutzfeld-Jakob disease (CJD). The results of the interview phase of the study were summarized in the Update sent to you in December of 1989. Since that time, the physicians conducting the study have analyzed the information relevant to the risk of CJD collected in the interview and from medical records. A paper summarizing this analysis will soon appear in the Journal of the American Medical Association.

Since we sent you the December 1989 Update, no new cases of CJD have been reported in recipients of NHPP growth hormone. The total number of cases in the United States remains at seven, out of nearly 6300 patients known to have received NHPP growth hormone. Unfortunately, outside the U.S. the number of cases of CJD associated with hGH has increased. There are now a total of six cases in England and one each in France, Brazil and New Zealand. The hormone used in Britain and France was completely unrelated to the U.S. hormone. The patients in Brazil and New Zealand received hormone prepared in the laboratories that produced growth hormone for the NHPP. Also, two patients in Australia developed CJD after treatment with other pituitary-derived hormones used for reproductive disorders.

Primates inoculated with samples of NHPP growth hormone have not developed CJD. These animals have now been monitored for over 5 years which is well beyond the 1-2 year minimum incubation period for CJD to be expressed in these animals.

No individual preparation of growth hormone has been identified from the study as the source of CJD. Because CJD has developed in patients treated with completely independent hormone preparations, we believe that multiple preparations were involved in the transmission of CJD. Most hormone preparations were given to a large number of people, yet only a small number of patients developed CJD. This suggests that levels of infectivity are very low and/or that susceptibility to CJD may vary considerably among patients. The major risk factor for developing CJD identified in the study is the length of time patients were treated with growth hormone. Those who developed CJD received growth hormone over an average period of about 8 years, which is more than twice the overall average length of treatment.

It is reassuring that there have been no new cases of CJD reported in the U.S. since 1988. It is possible that there may be some publicity about the upcoming medical report on the outcome of the epidemiological study. You should not be alarmed that any such press reports represent a new problem. Rather the information they will be reporting is the same information we have earlier provided to you through the Fact Sheet and Update. Although we cannot predict the total number of cases of CJD that will occur in growth hormone recipients, each year that passes without additional cases is a very hopeful sign.

We will continue to monitor recipients of NHPP growth hormone. The identifying information collected in the interviews will allow us to use national data on vital statistics to investigate deaths from any cause that may occur in these patients.

We will continue to provide you with information as it becomes available. In order to do this, it is important for us to have your current address. If the address to which this letter was sent is incorrect, please complete and return the enclosed change of address form. It is not necessary to return the form if your address has not changed.

As in the past, staff at the National Institute of Diabetes and Digestive and Kidney Diseases will continue to be available to answer questions you may have about this problem. You may contact:

National Institutes of Health
NIDDK Information Office
Building 31, Room 9A04
Bethesda, MD 20892
(301) 496-3583

Once again, I would like to take this opportunity to thank you for your participation in the epidemiology study and to express the continued interest of the Public Health Service in the well-being of all growth hormone recipients.

Sincerely,

Phillip Gorden, M.D.
Director

NOTE TO PHYSICIANS

The enclosed letter and fact sheet on human growth hormone (hGH) and Creutzfeldt-Jakob disease are being mailed to families of patients who have received pituitary hGH through the National Hormone and Pituitary Program. We have mailed this material to all families for whom we have current addresses. If you would like additional copies of the fact sheet to provide patients, please call (301) 496-3583.

National Institute of Diabetes
and Digestive and Kidney Diseases

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
National Institutes of Health

NOV 27 1987

National Institute of Diabetes and
Digestive and Kidney Diseases
Bethesda, Maryland 20892

Dear Growth Hormone Recipient:

I am writing to you because you or a member of your family received pituitary derived human growth hormone distributed by the National Hormone and Pituitary Program (NHPP). The National Institute of Diabetes and Digestive and Kidney Diseases, of which I am the director, provided support for the production and distribution of the hormone you received.

As you may know, distribution of pituitary human growth hormone (hGH) by both the NHPP and commercial companies halted in 1985 after three patients developed a rare, fatal brain disorder called Creutzfeldt-Jakob disease (CJD), which is caused by an agent similar to a virus. CJD has now affected a total of five American patients out of about 7,000 treated with NHPP hormone. These five cases show that CJD has occurred more frequently in people who received pituitary growth hormone than would be expected in the general population. We believe some of the growth hormone, derived from human pituitary tissue, was contaminated by the CJD agent. Many growth hormone recipients were given information about this problem in 1985 by their treating physicians, and I am enclosing current information with this letter.

Since the original recognition of this problem in 1985, we are making one new recommendation to patients who received pituitary growth hormone. Because there is no way of testing to detect infection with CJD, we are now recommending that pituitary growth hormone recipients not serve as blood or tissue donors. (This does not apply to recipients of biosynthetic growth hormone.) Physicians and blood bank officials who have considered this issue agree that the risk of transmission of CJD through blood transfusion from such patients is extremely small. In fact some studies showed that even when whole units of blood from patients with active symptomatic CJD were transfused into susceptible animals, there was no transmission of disease. However, other studies of transmission between animals suggest that there is a small chance of transmission through blood transfusion. Therefore, blood banks will not collect blood from anyone who has been treated with pituitary hGH. I want to emphasize that there is no risk to family members of patients and no special precautions are necessary with day-to-day family or social contact. Husbands and wives and other household members of patients with CJD have no higher risk of contracting the disorder than the general population. This new recommendation does not reflect any new information about CJD or growth hormone. It reflects instead the ever increasing vigilance of officials responsible for the nation's blood supply. Every possible measure is being taken to ensure the safety of the blood supply and to prevent even extremely remote risks.

I recognize the anxiety that patients treated with pituitary growth hormone and their families are experiencing, and I share your concern and unhappiness about this problem. We have tried to anticipate the questions

you might have about pituitary growth hormone and CJD and to answer them in the enclosed fact sheet. If you have particular concerns or questions that the accompanying information does not answer, your first source of information should be the physician who treated you or your child with pituitary growth hormone. If your treating physician is no longer available, my staff can arrange for a physician experienced about this problem to answer your questions. The number is (301) 496-3583.

For some questions you may have, we do not yet have complete answers. We are working to develop additional information that can be provided to patients, their families and physicians. One part of this effort is a study of the current health status of all patients who received pituitary growth hormone, which will be conducted over the next year. I will continue to provide you with new information about this problem as it becomes available.

Sincerely,

Phillip Gorden

Phillip Gorden, M.D.
Director

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
National Institutes of Health

National Institute of Diabetes and
Digestive and Kidney Diseases
Bethesda, Maryland 20892

January 31, 1994

Dear Growth Hormone Recipient:

I am writing to provide you with current information on the health status of the patients treated with growth hormone supplied by the National Hormone and Pituitary Program (NHPP). There have now been a total of eleven cases of Creutzfeldt-Jakob disease (CJD) among American growth hormone recipients. All of these patients began treatment before the newer method of pituitary-derived hormone preparation was introduced in 1977.

These eleven cases occurred among the approximately 8,000 patients who received NHPP growth hormone. That means that there has been about one case of CJD for every seven hundred people who received NHPP growth hormone. We wish we could predict exactly how many patients will eventually develop CJD, but unfortunately we cannot. The best information we can provide is that the rate of occurrence of CJD in the 8,000 NHPP growth hormone patients to date has averaged about one case per year since the problem was recognized in 1985.

Our follow-up study of growth hormone recipients was designed to identify other possible complications of growth hormone therapy. Several years ago there was a report from Japan that growth hormone recipients were at increased risk for leukemia. We asked about leukemia in the interview study, and have just analyzed and published our information. An increased risk of leukemia was found in NHPP growth hormone recipients who had brain tumors that caused their growth hormone deficiency. Four of the five patients with brain tumors who developed leukemia had received radiation therapy. There was no increase in leukemia among the great majority of NHPP growth hormone recipients who did not have brain tumors and radiation therapy as the cause of growth hormone deficiency.

We will continue to monitor all NHPP growth hormone recipients, and to provide you with information as it becomes available. Meanwhile, we would like to offer as much support for your concerns as we are able. I am providing responses to some questions that growth hormone recipients have asked in the hope that this information addresses your concerns. As in the past, staff at the National Institute of Diabetes and Digestive and Kidney Diseases are available to answer questions you may have about this problem. You may contact:

National Institutes of Health
NIDDK Information Office
Building 31, Room 9A04
Bethesda, MD 20892
(301) 496-3583

I would appreciate your taking the time to return the enclosed response card to Westat, Inc., the organization that maintains records for the Public Health Service on the follow-up study of growth hormone recipients. It is important that you notify us if your address has changed as well as about health problems you may have experienced, so we can continue to provide you with accurate information. We would particularly like you or your physician to contact us about any new or worsening neurologic symptoms or any diagnosis of leukemia. You may contact us at the address or phone number above or include a note with the enclosed response card.

Once again I would like to express the Public Health Service's concern for the well-being of all growth hormone recipients and assure you that we will keep you informed of any new developments.

Sincerely,

Phillip Gorden, M.D.
Director